

2020-02

# Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania

John, Betwel

NM-AIST

---

<https://dspace.nm-aist.ac.tz/handle/20.500.12479/1012>

*Provided with love from The Nelson Mandela African Institution of Science and Technology*

**PREFERRED RESTING SURFACES OF DOMINANT MALARIA  
VECTORS INSIDE DIFFERENT HOUSE TYPES IN RURAL SOUTH-  
EASTERN TANZANIA**

**Betwel John**

**A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of  
Master of Science in Public Health Research of the Nelson Mandela African Institution of  
Science and Technology**

**Arusha, Tanzania**

**February, 2020**

## ABSTRACT

Malaria control in Africa relies extensively on indoor residual spraying (IRS) and insecticide-treated nets (ITNs). Indoor residual spraying typically targets mosquitoes resting on walls, and in few cases, roofs and ceilings, using contact insecticides. Unfortunately, little attention is paid to where malaria vectors actually rest indoors, and how such knowledge could be used to improve IRS. This study investigated preferred resting surfaces of two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis*, inside four common house types in rural south-eastern Tanzania. The assessment was done inside 80 houses in south-eastern Tanzania across four villages. In each house, resting mosquitoes were captured using Prokopack aspirators from walls, undersides of roofs, floors, furniture, utensils, clothes and bed-nets. Overall, only 26% of *An. funestus* and 18% of *An. arabiensis* were found on walls. In grass-thatched houses, 33-55% of *An. funestus* and 43-50% of *An. arabiensis* rested under roofs, while in metal-roofed houses, only 16-20% of *An. funestus* and 8-30% of *An. arabiensis* rested under roofs. Considering all data together, approximately 40% of mosquitoes rested on surfaces not typically targeted by IRS, i.e. floors, furniture, utensils, clothes and bed-nets. These proportions were particularly high in metal-roofed houses (47-53% of *An. funestus*; 60-66% of *An. arabiensis*). While IRS uses contact insecticides to target adult mosquitoes on walls, and occasionally roofs and ceilings, significant proportions of vectors rest on surfaces not usually sprayed. This gap exceeds one-third of malaria mosquitoes in grass-thatched houses, and can reach two-thirds in metal-roofed houses. Where field operations exclude roofs during IRS, the gaps can be much greater. In conclusion, there is need for locally-obtained data on mosquito resting behaviours and how these influence the overall impact and costs of IRS.

**Keywords:** Malaria Control, Indoor Residual Spraying, Contact Insecticides, Malaria Vectors, *Anopheles funestus*, *Anopheles arabiensis*, Resting Behaviours.

## **DECLARATION**

I, Betwel John with registration number NM-AIST/M.400/T.17, hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation titled “Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania” is my original work under the guidance of the supervisor listed below. It is being submitted in partial fulfilment of degree of Master of Science in Public Health Research to the Nelson Mandela African Institution of Science and Technology and it has not been submitted to any other learning institution.

-----

Betwel John

-----

Date

## **COPYRIGHT**

This dissertation is copyright material protected under the Berne Convention, the Copyright Act of 1999 and other international and national enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review or discourse with an acknowledgement, without the written permission of the office of Deputy Vice Chancellor for Academics, Research and Innovation on behalf of both the author and Nelson Mandela African Institution of Science and Technology.

## CERTIFICATION

I, undersigned have read dissertation titled “Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania”, submitted by Betwel John in partial fulfillment of the requirements for the degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology, I hereby confirm that it is an authentic work and recommend for its acceptance by the Institute.

-----  
Fredros Okumu, PhD  
Adjunct Professor, NM-AIST  
**Supervisor**

-----  
Date

## ACKNOWLEDGEMENTS

The journey of this research began two years ago, in which period I have been supported by several people. For that, I must acknowledge the contribution of all who helped in one way or another to ensure objectives of this study were achieved. First, I would like to thank God Almighty for the strength and protection throughout this entire study.

As a Master's student I met Dr. Fredros Okumu, who recognized me and took me as his student although I had little understanding of mosquitoes, and the field of entomology in general. Under his supervision, I have been able to learn a lot in the field entomology and beyond. Thus, I am directing my deepest gratitude to my supervisor, Dr. Okumu for his assistance, patience, time and scientific input throughout the time I spent with him. His supervision has greatly fostered my thinking and provided me with enabling environment for my personal and career development. For that, thank you so much Dr. Fredros Okumu.

I extend my gratitude to research team members of outdoor mosquito control (OMC) at Ifakara Health Institute: Dr. Emmanuel W. Kaindoa, Mr. Halfan Ngowo, Ms. Najat Kahamba, Mr. Dickson Msaky, Mr. Anord Mmbando, Ms. Marcelina Finda, Mr. Ismail Nambunga, Ms. Rukiyah Mohammed, Ms. Doreen Josen Siria, Mr. Issa Mshani, Mr. Pinda Polius, Mr. Alex Limwagu, Mr. Emmanuel Happe and Ms. Elihaika Minja. The team has been there to provide me with encouragement and support in times of difficulties as well as with scientific inputs, analysis planning and execution, in reviewing study protocols and proofreading reports of this study, to mention a few. I also specifically thank Mr. Japhet Kihonda for technical support during field collection, processing and morphological identification of mosquitoes.

Also, I would like to acknowledge administrations of Kilombero and Ulanga District Councils for allowing us to work in their administrative area. I also thank community of Kilombero and ulanga for letting us work in their houses. I also acknowledge volunteers who helped us in engaging the community, Mr. Hassan Ngege, Mr. Kado Ligawandu, Mr. Mussa Kinjengalile, Mr. Maxcelin Ngakuka. I especially thank Mr. Hamza Nambongo, Mr. Kilumite Nambongo, Mr. Bakari Rajabu, Mr. Zakaria Ngelela and Mr. Khalid Kaulawa for helping in collection of mosquitoes in their houses during night times.

During my study period, my family has been very close to me and given me the support I needed. They were committed to ensure that I get good education and acted as a catalyst in making me work hard when I faced challenges. Thus, my warm appreciation goes to my parents, John Jonathan Msugupakulya and Atu Jacob Mwambene for their encouragement and support throughout, and to my siblings; Janeth John Msugupakulya and Japhet John Msugupakulya.

Also, special credit to my classmates, I thank them all for their support, and valuable hints that have been part and parcel of accomplishment of this study. Without forgetting special thanks to all individuals who helped in diverse ways in making this work a success, I am most grateful.

This study was supported by Ifakara Health Institute (Training and Capacity Building Department) as part of the Master of Science Studentship awarded to Betwel John, and Howard Hughes Medical Institute – Gates International Research Scholarship awarded to Dr. Fredros Okumu (Grant No. OPP1099295). I am grateful to both funding partners.



## **DEDICATION**

I dedicate this work to my family and friends.

## Table of Contents

ABSTRACT .....	i
DECLARATION .....	ii
COPYRIGHT .....	iii
CERTIFICATION .....	iv
ACKNOWLEDGEMENTS .....	v
DEDICATION .....	vii
LIST OF TABLES .....	x
LIST OF FIGURES .....	xi
ABBREVIATIONS .....	xii
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.1. Background of the problem .....	1
1.2. Statement of the problem .....	2
1.3. Rationale of the study .....	3
1.4. Research objectives.....	4
1.4.1. Main objective .....	4
1.4.2. Specific objectives .....	4
1.5. Research questions.....	4
1.6. Significance of the study.....	4
1.7. Delineation of the study .....	5
CHAPTER TWO .....	6
LITERATURE REVIEW .....	6
2.1. Vectors of malaria in Africa .....	6
2.2. Malaria control tools .....	6
2.3. Feeding preferences of malaria vectors and implications in malaria interventions .....	7
2.4. Resting behaviours of malaria vectors and house spraying with insecticides .....	7
CHAPTER THREE .....	10
MATERIALS AND METHODS.....	10
3.1. Study area.....	10
3.2. Selection and characterization of study houses .....	11
3.3. Collection of mosquitoes resting on different surfaces inside the houses .....	12
3.4. Morphological identification and processing of collected mosquitoes.....	13

3.5. Identification of sibling species of malaria vectors, blood meal analysis and detection of <i>Plasmodium falciparum</i> sporozoites in the mosquitoes.....	13
3.6. Determination of physiological ages of mosquitoes .....	14
3.7. Data analysis .....	14
3.8. Ethics approval and consent to participate.....	15
CHAPTER FOUR.....	16
RESULTS AND DISCUSSION .....	16
4.1. Results.....	16
4.1.1. Descriptive summary of mosquitoes caught in the surveys .....	16
4.1.2. Resting preferences of mosquitoes inside the houses .....	16
4.1.3. Effects of household variables on preferences of mosquitoes for different resting surfaces inside houses. ....	26
4.1.4. <i>Anopheles</i> sibling species and <i>Plasmodium</i> infections .....	28
4.1.5. Mosquito blood meal sources and parity statuses .....	28
4.2. Discussion .....	29
CHAPTER FIVE .....	33
CONCLUSION AND RECOMMENDATIONS .....	33
5.1. Conclusion .....	33
5.2. Recommendations.....	33
REFERENCES .....	35
RESEARCH OUTPUTS .....	46
Output 1: Published paper.....	46
Output 2: Poster presentation.....	46

## LIST OF TABLES

Table 1: Numbers and percentages of mosquitoes of different species collected from different surfaces inside houses of different types .....	17
Table 2: Summary statistics for comparison of the number of mosquitoes of different species collected from walls, roofs and other surfaces inside the different house types.....	19
Table 3: Numbers and percentages of mosquitoes of different species collected from surfaces typically not targeted by IRS inside different houses types.....	22
Table 4: Relationship between of household risk factors and indoor temperatures on mosquito resting preference on different surfaces .....	27

## LIST OF FIGURES

Figure 1: Map showing study villages and study households in both Kilombero and Ulanga districts, south-eastern Tanzania.....	11
Figure 2: Typical house types in the study villages in rural south-eastern Tanzania.....	12
Figure 3: Overall nightly densities of malaria vectors <i>Anopheles funestus</i> and <i>Anopheles arabiensis</i> , from different resting surfaces inside the houses .....	20
Figure 4: Overall densities of <i>Culex</i> mosquitoes, from different resting surfaces in houses.....	21
Figure 5: Comparison of <i>Anopheles funestus</i> densities on different resting surfaces in different house types.....	23
Figure 6: Comparison of <i>Anopheles arabiensis</i> densities on different resting surfaces in different house types.....	24
Figure 7: Comparison of densities of <i>Culex</i> mosquitoes, from different resting surfaces in different house types .....	25

## **ABBREVIATIONS**

ACT	–	Artemisinin-based Combination Therapy
AIC	–	Akaike Information Criterion
ELISA	–	Enzyme-Linked Immunosorbent Assay
GLMM	–	Generalized linear mixed effects models
IHI	–	Ifakara Health institute
IRS	–	Indoor Residual Spraying
ITNs	–	Insecticide-Treated Nets
LLINs	–	Long-Lasting Insecticide-treated Nets
NBS	–	National Bureau of Statistics
NIMR	–	National Institute for Medical Research
PCR	–	Polymerase Chain Reaction
PMI	–	President's Malaria Initiative
RR	–	Rate Ratio
WHO	–	World Health Organization

## CHAPTER ONE

### INTRODUCTION

#### 1.1. Background of the problem

Malaria control efforts have yielded significant success in recent decades, resulting in decline in number malaria cases from 251 million in 2010 to 228 million in 2018 (World Health Organization (WHO), 2019b). The most widely used interventions, namely insecticide-treated nets (ITNs), indoor residual spraying (IRS) and artemisinin-based combination therapy (ACT) are credited with 663 million clinical cases of malaria averted between 2000 and 2015 (Bhatt *et al.*, 2015). In Tanzania, the impact of these interventions has been demonstrated by multiple investigators (Alba *et al.*, 2014; Bhattarai *et al.*, 2007; Mashauri *et al.*, 2013; Selemani *et al.*, 2016), as well as national surveys, which show significant overall reduction in burden (National Bureau of Statistics (NBS) *et al.*, 2018). Despite these gains, there is also evidence that the anti-malaria progress is levelling off and that the gains may be lost (WHO, 2018b). Between 2015–2017, continued utilization of the core interventions led to no significant declines in malaria at global scale (WHO, 2018b).

To rejuvenate the malaria fight, several countries have set ambitious goals in line with the WHO Global Technical Strategy for Malaria Elimination (WHO, 2015b), and more recently, the High Burden to High Impact initiative which targets the ten most malarious countries in Africa, plus India (WHO, 2018a). The new initiatives are expected to be much more aggressive and country-led but involving multiple partners. However, similar to previous efforts, these efforts are primarily reliant on ITNs (now long-lasting insecticide-treated nets (LLINs)), IRS and effective case management (WHO, 2019b). Despite proven effectiveness of the vector control interventions, LLINs and IRS are negatively affected by insecticide resistance (Hemingway *et al.*, 2016; Ranson & Lissenden, 2016), increasing outdoor-biting (Finda *et al.*, 2019; Monroe *et al.*, 2019; Sherrard-Smith *et al.*, 2019), high costs and the sub-optimal coverage and usage at community and household level. Resistance is often associated with exposure of vectors to insecticides used in agriculture (Nkya *et al.*, 2014) and public health (Protopopoff *et al.*, 2008; Stump *et al.*, 2004), and the indoor interventions may also induce shifts in vector biting and resting behaviours (Killeen & Chitnis, 2014; Moiroux *et al.*, 2012; Russell *et al.*, 2010).

## 1.2. Statement of the problem

IRS is one of the oldest malaria interventions and was the most important component of the initial attempts at global malaria eradication in 1950s and 1960s (Kouznetsov, 1977; Nájera *et al.*, 2011). It involves applying insecticides to kill mosquitoes resting on interior walls of houses (WHO, 2015a). In Tanzania, it has been used intermittently since the 1960s (Pringle, 1967), and is currently deployed in selected districts mostly in the northern regions where malaria burden remains very high (National Malaria Control Programme, 2014; The Presidents Malaria Initiative (PMI) VectorLink Project, 2019). Across Africa, IRS is mostly promoted by the United States (US) Presidents Malaria Initiative, and currently covers 14 countries in Africa (U.S. President's Malaria Initiative, 2019b, 2019a). According analysis by Bhatt *et al.* (2015) IRS alone contributed to 10% of averted clinical malaria cases in Africa between 2000 and 2015 (Bhatt *et al.*, 2015). To counter the growing challenge of insecticide resistance (WHO, 2012), most countries have switched from using pyrethroids, and now rely mostly on organophosphates or carbamates, as well as some new insecticide classes such as neonicotinoids, which were recently introduced (WHO, 2019a). There have also been calls to introduce bed-nets with multiple active ingredients or synergists as a way to tackle resistance (Gleave *et al.*, 2018; Protopopoff *et al.*, 2018; Tiono *et al.*, 2018).

While much of the focus is paid to finding new chemical actives and combinations, considerably less attention is paid to how malaria mosquitoes actually respond to the indoor interventions such as IRS and LLINs. This is despite the changing housing designs and structures across Africa (Tusting *et al.*, 2019), and the demonstrated impact of housing on vector densities and malaria transmission (Kirby *et al.*, 2013; Lindsay *et al.*, 2003; Tusting *et al.*, 2015, 2017). Instead indoor interventions still primarily rely on historical evidence of mosquito indoor resting habits (Smith, 1962b, 1962a), which are now due for update in light of modern transformations (Tusting *et al.*, 2019). A study from early 1960s in Tanzania assessed distribution of malaria vectors on sprayable surfaces inside houses compared to household possessions usually removed during IRS (Smith, 1962b). It was observed that less than 20% of mosquitoes rested on the possessions, and that of the remaining, sprayable surfaces, the resting populations were evenly divided between substrates (Smith, 1962b). In a separate study in mud huts in northern Tanzania, 56% to 70% of all resting



mosquitoes were found on the walls or hanging articles, while the remaining 30% to 40% were on the underside of the roofs (Smith, 1962a).

Other than these early studies, such investigations have become rare, yet it is likely that mosquito behaviours and survival inside houses could change with the ongoing improvements. For example, a recent study in the Gambia demonstrated that reduced mosquito survival in metal-roofed houses may lower malaria transmission (Lindsay *et al.*, 2019). Elsewhere in East Africa, it was shown that despite higher temperatures inside houses with corrugated iron roofs, survival of mosquitoes resting indoors was same as in grass thatched houses (Okech *et al.*, 2004).

It is, therefore, crucial to understand resting behaviours of the major malaria vectors inside houses and how much they can be affected by key indoor interventions. This way, effectiveness of techniques such as IRS can be improved, and their limitations determined. This study therefore investigated the resting behaviours of two major malaria mosquitoes (*An. funestus* and *An. arabiensis*) inside typical house types in rural south-eastern Tanzania. In this area, most malaria infections are mediated by *An. funestus*, even though *An. arabiensis* remains abundant as well (Kaindoa *et al.*, 2017; Lwetoijera *et al.*, 2014).

### **1.3. Rationale of the study**

In recent years anti-malaria progress has been approaching steady rate (WHO, 2018b), partly because existing interventions, are negatively affected by multiple challenges such as insecticide resistance (Hemingway *et al.*, 2016; Ranson & Lissenden, 2016), high costs and the sub-optimal coverage and usage at community and household level. This, therefore calls for alternative approach to control malaria and enhancing existing interventions to bring the fight against malaria back on track.

Indoor residual spraying has been used for many years to control malaria and was one of the key interventions during early malaria eradication in 1950s to 1960s (Kouznetsov, 1977; Nájera *et al.*, 2011). It involves spraying insecticides on the walls of houses, and occasionally on roofs. Unfortunately, current procedures were developed several decades ago, when houses in Africa were still mostly thatch-roofed. Surprisingly, this practice has not changed despite improvements in housing across Africa in recent years (Tusting *et al.*, 2019). Therefore, it was important to do this research to learn on indoor resting behavior of mosquito in response to current housing. This

knowledge is important in the fight against malaria, as it can be used in our advantage to ensure we achieve better result in the battle against malaria mosquitoes. For example, information can be used to enhance effectiveness of house spraying as it shows where exactly we can find more mosquitoes, making it easier to introduce strategies in house spraying to achieve the great impact against malaria vectors.

## **1.4. Research objectives**

### **1.4.1. Main objective**

To investigate indoor resting behaviours of mosquito vectors and factors influencing their indoor resting preferences in rural south-eastern Tanzania.

### **1.4.2. Specific objectives**

- (i) To assess preferred indoor resting surfaces of major malaria vectors inside different house types in south-eastern Tanzania.
- (ii) To assess associations between household factors and indoor resting preferences of mosquitoes.

## **1.5. Research questions**

- (i) What are the preferred indoor resting surfaces of major malaria vectors inside house?
- (ii) What are the associations between household factors and indoor resting preferences of mosquitoes?

## **1.6. Significance of the study**

This research created baseline knowledge on indoor resting behaviour of the major malaria vectors inside human occupied houses. Indoor residual spraying, an intervention that target resting mosquitoes involves targeting mosquitoes resting on walls however one third to two third of malaria vectors rest on surfaces that are not targeted by IRS. Thus, the knowledge generated in this study will help in planning and improving interventions targeting resting mosquitoes (especially IRS) to accelerate control and elimination efforts of residual malaria transmission.

### **1.7. Delineation of the study**

Initially when this study was set up, collections of mosquitoes were done in the morning. This resulted to most collections of mosquitoes to be carried in the morning. It is during this time when household members participate in household chores. Thus, choice of mosquitoes to rest during morning time might have been influenced by human movement in the morning. Thus, collections in the morning might have underestimated mosquitoes resting on surfaces such as floors, furniture and utensils.

These observations of indoor resting behaviours of mosquitoes were carried out in villages that are not protected with IRS. However, evidences have shown that mosquitoes tend to change behaviour as a response to interventions. Therefore, it is important that future studies should be carried-out to assess indoor resting preference of mosquitoes in houses protected with IRS.

This study did not track movement of mosquitoes inside house rather observed where they were during time of collection. Therefore, it is acknowledged that proportion of mosquitoes resting on surfaces other than walls does not mean that these mosquitoes would never come into contact with walls or would not be killed by IRS when walls are sprayed. For this reason, additional studies may be required to examine how these resting preferences of mosquitoes inside houses impact effectiveness of IRS.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1. Vectors of malaria in Africa

In most African regions, major vectors of malaria comprise *Anopheles* mosquitoes belonging to *Anopheles funestus* group (*An. funestus* s.s.) and *Anopheles gambiae* complex (*An. gambiae* s.s., *An. arabiensis* and *An. colluzzi*) (Coetzee *et al.*, 2013; Sinka *et al.*, 2012). Both *An. funestus*, *An. gambiae* and *An. arabiensis* are dispersed across settings in western, eastern, southern, and central Africa (Sinka *et al.*, 2012). *Anopheles arabiensis* is outspreading further to arid and semi-arid areas of Africa such as Ethiopia and northern parts of Botswana and Namibia as well as in Sahel region (Sinka *et al.*, 2012). *Anopheles colluzzi* on the other hand extends from West Africa to Central Africa and some parts of east Africa, where samples of *An. colluzzi* identified in parts of Zimbabwe (Coetzee *et al.*, 2013).

*Anopheles gambiae* have historically been most important vector among major malaria vectors, in several settings in Africa. However, its population has been reduced to undetectable levels (Bayoh *et al.*, 2010; Lwetoijera *et al.*, 2014). In these region *An. funestus* now presents major threats despite occurring in fewer numbers compared to *An. arabiensis*. For example, in villages of south-eastern Tanzania *An. funestus* are credited for mediating nearly nine out of 10 malaria cases in that region (Kaindoa *et al.*, 2017).

#### 2.2. Malaria control tools

Malaria control and elimination relies on tools such as insecticide treated nets, indoor residual spraying (vector control tools), effective case management and intermittent preventive treatment during pregnancy (WHO, 2018b). Insecticide treated bed-nets (ITNs) and indoor residual spraying (IRS) are regarded as important vector control strategy for malaria control and elimination, targeting indoor biting and indoor resting mosquitoes. They have demonstrated impressive impact in malaria control and elimination. According to Bhatt *et al.* (2015) among 663 million cases averted in Africa between 2000 and 2015, 68% were contributed by ITNs and 10% by IRS (Bhatt *et al.*, 2015). Apart from epidemiological impact of these intervention, as mentioned earlier these

tools have resulted to significant reduction of the competent vector, *An. gambiae* s.s. in several regions of Africa (Bayoh *et al.*, 2010; Lwetoijera *et al.*, 2014).

### **2.3. Feeding preferences of malaria vectors and implications in malaria interventions**

Vector control and elimination efforts are partly affected by preference of mosquitoes to feed indoor or outdoor. Whereby, mosquitoes that prefer obtain blood meal outside houses (exophagic) are less likely to be affected by indoor interventions. For example, *An. gambiae* s.s. and *An. funestus* s.s. represent malaria vectors with higher preference of indoor feeding (endophagic) (Pates & Curtis, 2005). Indeed, it is because of this behaviour, expansion of indoor interventions such as insecticide treated bed-nets has led to untraceable numbers of *An. gambiae* s.s. (Bayoh *et al.*, 2010; Lwetoijera *et al.*, 2014). However, *An. arabiensis* represents malaria vectors with higher degree of exophagic behaviour. *Anopheles arabiensis* thrives even in areas with high coverage indoor interventions, feeding on human when outdoor as well as on other animals when humans are absent or protected (Tirados *et al.*, 2006). Thus, outdoors feeding mosquitoes evade fatal incidents with indoor interventions such as long-lasting insecticides treated nets and indoor residual spraying.

### **2.4. Resting behaviours of malaria vectors and house spraying with insecticides**

Once mosquitoes obtain blood meals, they can either rest inside or outside houses for digestion of blood and development of eggs. Earlier study demonstrated that mosquitoes went indoor to obtain blood where they also rest until they are ready to lay eggs (De Meillon, 1934). House spraying utilizing this behaviour have been successful in several setting, and was the primary vector control tool during the Malaria eradication 1955-1969 (Kouznetsov, 1977; Nájera *et al.*, 2011).

In control of mosquito vector using indoor interventions, it is important to understand if mosquitoes prefer to rest inside houses and to determine where exactly mosquitoes prefers to rest when they are inside human dwellings. In historical times, some studies have gone beyond indoor-outdoor resting behavior of mosquitoes and assessed where exactly malaria vectors rest inside houses. For example, in villages of Ukara Island in Lake Victoria of Tanzania, a study in huts made of mud walls and thatched roofs found that less than 40% of *An. funestus* and *An. gambiae* were resting on roofs. Percentage of mosquitoes found resting on surfaces below roof level was

between 61% and 70% (Smith, 1955). In a separate study in Ubugwe, northern Tanzania, daytime collections made between 1959-1960 in mud huts with turf or thatched roofs, similar results were observed, 56% to 70% of *An. gambiae* were found resting on walls or hanging articles, while from 30% to 44% *An. gambiae* were found resting underside of roofs (Smith, 1962a). In another study from early 1960s in Tanzania assessed distribution of malaria vectors on sprayable surfaces inside houses relative to non-sprayable household possessions (Smith, 1962b). It was observed that less than 20% of mosquitoes rested on the possessions that are usually removed during IRS, and that of the remaining sprayable surfaces, resting mosquitoes were evenly divided between surfaces (Smith, 1962b).

Similarly, in earlier studies in Kenya and Uganda, resting preference of African malaria vectors was shown to be more on higher surfaces such as roof or ceiling and upper parts of walls over floor and other resting surfaces (Gibbins, 1933; Haddow, 1942). Apart from these studies in East Africa, resting behavior of mosquitoes inside houses was also investigated in a study in villages of Burkina Faso (West Africa). The purpose of this study was to assess dichlorvos using evaporators as a residual fumigant for malaria control in mud houses. In this study, it was observed that 94.6% *An. funestus* and *An. gambiae* were rested on ceiling (Mathis *et al.*, 1963).

Elsewhere beyond Africa, indoor resting behavior of different species of malaria vectors have also been documented. A study by Ogata in 1990's documented preferences of *Anopheles albimanus* inside houses with thatched roofs and woody or bamboo walls. In this study mosquitoes were observed to have higher preference of resting on roofs. Whereby 53% of mosquitoes were observed resting on underside of roofs, 28.3% on walls, 13.4% on furniture, 2.1% ceiling, 2.1% on eaves, and 1.1% on exterior walls (Ogata *et al.*, 1992). In east central India, resting preference of *Anopheles fluviatilis* and *Anopheles minimus* were studied. The two species had exclusive preference of resting on walls. Of all *An. fluviatilis* collected indoor 99.3% were resting on walls and 0.7% were resting on hanging objects, while all of *An. minimus* were resting on walls (Sahu *et al.*, 2011). On walls, large percentage of both species were concentrated on portion of walls (3-4 ft from the floor), ranging between 45.6% and 47.3%. Below 3 ft and above 4 ft the percentage of mosquitoes were between 21.6% and 32.8% (Sahu *et al.*, 2011). A more recent study in area with houses with stone/brick walls and stone slab/concrete roofs, *Anopheles stephensi* were observed to have higher preference of resting on non-sprayable household items such as

cupboards, furniture, clothes, stored goods, cobwebs and floor. Percentage of mosquitoes resting on household items was between 95% and 97% while less than 5% rested on sprayable surfaces such as walls, roofs and windows (Nagpal *et al.*, 2012).

It appears that resting preferences of mosquitoes inside houses varies between mosquito species and geography. Thus, it is important to assess resting preferences of mosquito species in different geography rather than relying on historical evidences. Unfortunately, other than these early studies, there have been few attempts to examine mosquito resting preferences indoors and how this may influence vector control efforts such as IRS.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1. Study area

The study was conducted in four villages across Ulanga and Kilombero districts in south-eastern Tanzania (Fig. 1). These included, Kivukoni (-8.2021, 36.6961) and Tulizamoyo (-8.3669, 36.7336) in Ulanga district, and Sululu (-7.9973, 36.8317) and Ikwambi (-7.9833, 36.8184) in Kilombero district. The area is within a low-lying river valley extending 250 km long and up to 65 km wide, interspersed with villages and farmlands. It has two rainy seasons, short rains between November and December and long rains between March and May, while between rainy seasons spans two dry seasons. Annual rainfall and temperatures vary from 1200 mm to 1800 mm, and 16°C to 32°C, respectively (World Weather Online, 2019). Residents are mostly subsistence farmers, though some are also fishermen or owned small businesses.

During this study, typical house types in the villages were either thatch-roofed or metal-roofed (with corrugated iron sheets), and had either mud walls or brick walls, which were sometime plastered with concrete. Primary malaria vectors in this region are *An. funestus* and *An. arabiensis*, with *An. funestus* contributing more than 80% of current malaria transmission (Kaindoa *et al.*, 2017). *Culex pipiens* are nuisance biters contributing 79% of all indoor biting risk (Matowo *et al.*, 2019).



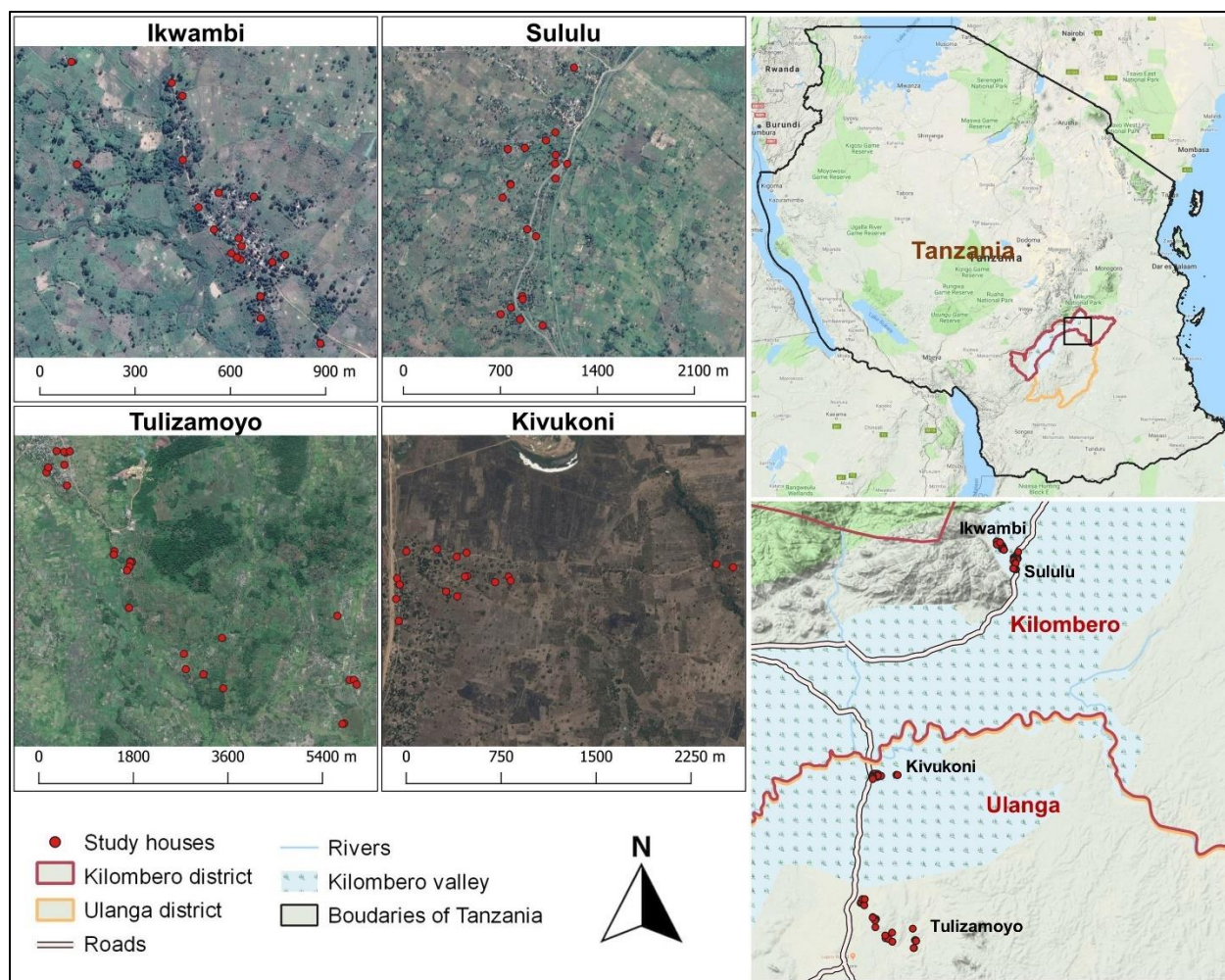


Figure 1: Map showing study villages and study households in both Kilombero and Ulanga districts, south-eastern Tanzania

### 3.2. Selection and characterization of study houses

Field collection of resting mosquitoes was done inside human-occupied houses, ensuring to cover the main house types. Candidate houses were selected based on construction materials for walls (mud or bricks, with or without concrete plastering) and roofs (metal or thatch). This resulted in four classes of houses (Fig. 2) commonly found in the study area, namely: (a) houses with thatched roofs and mud walls, (b) houses with thatched roofs and brick walls (none of these houses had plastered walls), (c) houses with metal roofs and un-plastered brick walls, and (d) houses with metal roofs and plastered brick walls. Ceilings were uncommon and therefore excluded in this survey. All individual houses were also geo-referenced, then characterized by other attributes, namely: (a) whether eave gaps were open or closed, (b) number of rooms in the house, (c) height

of walls and (d) maximum daily temperatures ( $^{\circ}\text{C}$ ), recorded using Tinytag® data loggers (Gemini, UK) hung at height more than a meter from the roof, or on a dry surface away from the floor.

A sample size of 20 houses was estimated using the Cohen simulation with the pwr R package, to achieve at least 80% power with 0.5 effect size. Therefore, prior to commencement of mosquito collections, 20 houses were purposively selected in each of the four villages upon consent by household heads. These included five houses per house type.

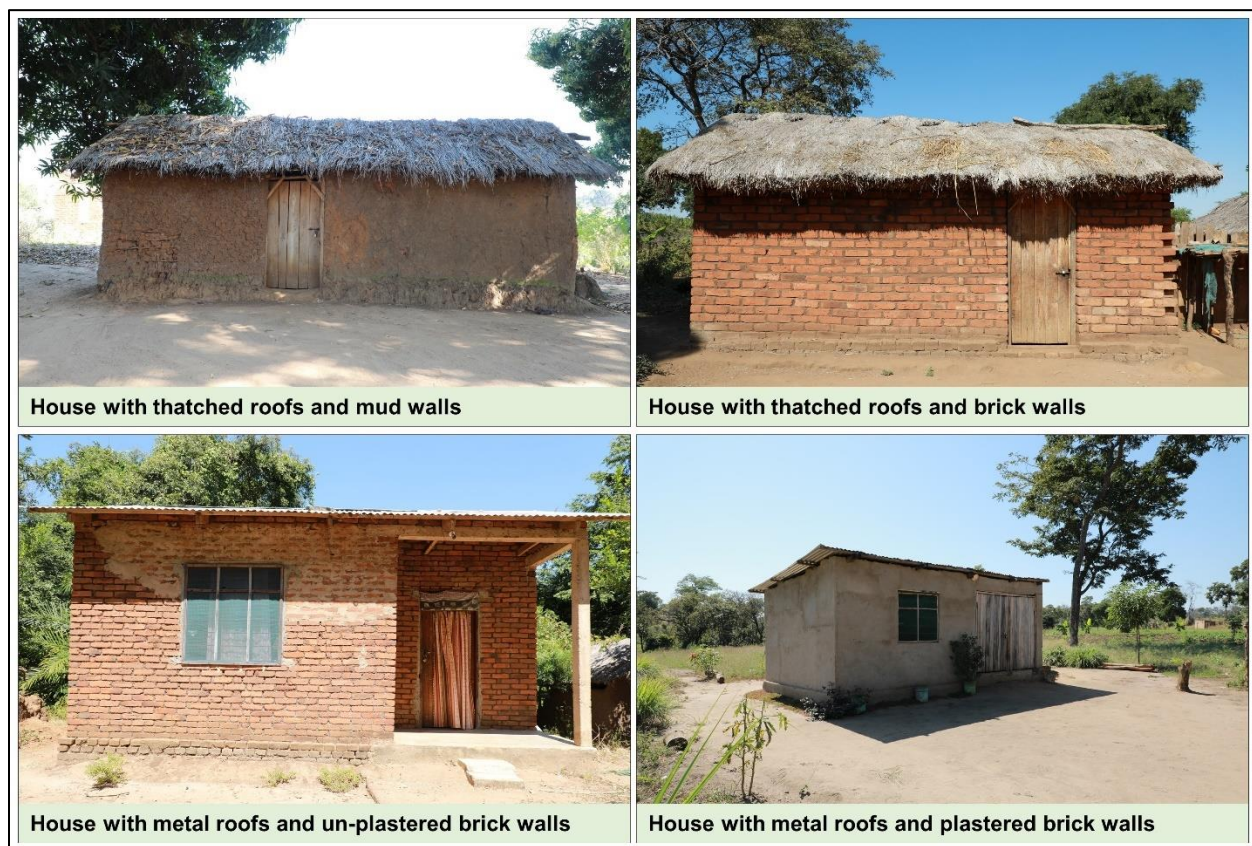


Figure 2: Typical house types in the study villages in rural south-eastern Tanzania

### 3.3. Collection of mosquitoes resting on different surfaces inside the houses

Potential mosquito resting places were identified to include: (a) walls, (b) roofs (underside of the roofs) and (c) other surfaces such as floor, clothing, bed-nets and other household items. The household items were varied but generally included furniture such as beds, tables, chairs, cupboards, wood blocks, other household items such as bicycles, and utensils, wash basins, water containers, clay pots and cooking pans. The clothing included hanging garments, curtains, sacks and bags. Actual mosquito collections were done using Prokopack aspirator (Maia *et al.*, 2011) by

trained technicians. Collections involved hovering the aspirator systematically over the surfaces and collecting all mosquitoes. Lighting was provided using hand-held flash lights.

The sequence of collection between resting surfaces in each room was changed to minimize sampling biases. The collections were done for five days each week in each village, visiting 2-4 houses per day. Initially the collections were done between 0600 h and 12 h, from January 2019 to May 2019. Then from May to July 2019, the collections were done three times a day (in the morning (between 0700 h and 0830 h), evening (between 1800 h and 2000 h) and at night (between 2400 h and 0200 h)), to minimize variations associated with mosquitoes moving between different resting surfaces within the houses. Unlike the other collections done by trained technicians, the late evening and late-night collections were done by trained household members to avoid intrusion of their privacy.

In total, there were 277 house visits for indoor resting mosquito collections, including 76 visits to houses with thatched roofs and mud walls, 70 to houses with thatched roofs and brick walls, 70 to houses with corrugated iron roofs and un-plastered brick walls, and 61 visits to houses with corrugated iron roofs and plastered brick walls.

### **3.4. Morphological identification and processing of collected mosquitoes**

Mosquitoes collected from each of the resting surfaces were placed in separate disposable cups and labelled appropriately. They were sorted by sex and taxa, then all *Anopheles* sorted and identified using the morphological keys (Gillies & Coetzee, 1987). Physiological status of each female *Anopheles* was determined as unfed, partly fed, fully fed, gravid or semi gravid. All records were kept by house, surface, house type and village.

### **3.5. Identification of sibling species of malaria vectors, blood meal analysis and detection of *Plasmodium falciparum* sporozoites in the mosquitoes**

The field-collected mosquitoes were packed individually in 1.5 ml microcentrifuge tubes (BioPointe Scientific®) containing silica plugged with cotton wool. Sub-samples of *An. funestus sensu lato* (s.l.) and *An. gambiae* s.l females were further analysed for sibling species, *Plasmodium falciparum* sporozoites and blood meal sources (if the mosquitoes were blood-fed). Sibling species identification for *An. funestus* s.l and *An. gambiae* s.l was done using polymerase chain reaction



(PCR) protocols originally developed by Koekemoer and others (Koekemoer *et al.*, 2002) and Scott and others (Scott *et al.*, 1993) respectively. Blood meal analysis was done using enzyme-linked immunosorbent assay (ELISA) tests (Beier *et al.*, 1988), and parasite infections detected by screening for the *P. falciparum* circumsporozoite proteins in salivary glands of the adult females (Wirtz *et al.*, 1987). Heat-labile non-*P. falciparum* were eliminated by boiling the ELISA lysates at 100°C for 10 minutes to remove false positives (Durnez *et al.*, 2011).

### **3.6. Determination of physiological ages of mosquitoes**

Parity of mosquitoes was approximated following procedure described by Detinova (Detinova, 1962) as a proxy of physiological age of mosquitoes. A subsample of non-blood fed, *An. funestus* and *An. arabiensis*, were first immobilized in a refrigerator. Under stereo microscope abdomens of anesthetized mosquitoes were dissected to extract ovaries. Ovaries were examined under compound microscope to determine whether mosquitoes had laid eggs or not.

### **3.7. Data analysis**

Data analysis was done using open source statistical software, R version 3.6.0 (R Core Team, 2019). Generalized linear mixed effects models (GLMM) were built using functions within the *lme4* package (Bates *et al.*, 2015) to assess: (a) preferences of mosquitoes (*An. funestus*, *An. arabiensis* and *Culex*) for different resting surfaces and (b) relationships between various household risk factors and number of mosquitoes caught on different surfaces. Initially, the number of female mosquitoes of each species was modelled as a response variable against resting surfaces as a fixed factor. Since walls are typically the main target for insecticide spraying, they were used as reference against which other surfaces were compared.

To assess relationships between household risk factors and mosquitoes resting on different surfaces, the number of mosquitoes caught from each surface was modelled as function of: (a) roof type, (b) wall type, (c) whether interior walls were plastered with cement or not, (d) eave gaps, (e) number of rooms, (f) wall height and (g) daily maximum temperatures inside the houses.

In all models, households nested within villages and sampling days were used as random terms, to capture unexplained variations, and account for pseudo-replication. Poisson distribution was used when fitting GLMM models, except when overdispersion was detected, in which cases, negative

binomial distribution was used instead. The best fitting models were selected using Akaike Information Criterion (AIC) (Bolker, 2007), and results presented as relative rate ratios (RR) at 95% confidence intervals. In addition, the *dabestr* package for estimation statistics (Ho *et al.*, 2018), was used to depict effect sizes of differences in mean numbers (at 95% confidence intervals) of mosquitoes collected on different resting surfaces relative to walls.

### **3.8. Ethics approval and consent to participate**

Detailed explanations on aim, procedures, potential risks and benefits of the study was provided to household occupants before study commencement. Written informed consent in local language (*Swahili*) were obtained from household heads before inclusion in the surveys. Ethical approval for the study was granted by Institutional Review Board of Ifakara Health Institute (IHI/IRB/No: 007-2018) and the Medical Research Coordination Committee of the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol.IX/2895).

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1. Results

##### 4.1.1. Descriptive summary of mosquitoes caught in the surveys

A total of 17 870 female mosquitoes were collected, of which 31.1% (n= 5 564) were *Anopheles* mosquitoes and 68.9% (n=12 306) were culicines. Among *Anopheles* mosquitoes, 81.5% (n=4535) were *An. funestus* s.l, 17.6% (n= 977) were *An. arabiensis* and 0.9% (n=52) were other *Anopheles* species including *Anopheles custani* and *Anopheles pharoensis*. The majority of *An. funestus* (72.4%), *An. arabiensis* (87.8%) and *Culex* (58.0%) were collected in thatch-roofed houses.

##### 4.1.2. Resting preferences of mosquitoes inside the houses

There was an uneven distribution of mosquitoes between the four house types and between the different resting surfaces (Tables 1 & 2; Fig. 3). Only 26.1% of *An. funestus*, 18.2% of *An. arabiensis* and 27.9% of *Culex* mosquitoes rested on walls. Proportions resting on the undersides of the roofs included 32.9% of *An. funestus*, 42% of *An. arabiensis* and 33.6% of *Culex* mosquitoes. Surprisingly, as many as 41% *An. funestus*, 40% of *An. arabiensis* and 39% of *Culex* mosquitoes rested on surfaces other than either the walls or roofs, i.e. surfaces that are not typically sprayed during IRS. The actual distribution of the two malaria vector species and the *Culex* mosquitoes also depended on house construction materials. Nearly 80% of *An. funestus* and *An. arabiensis* were collected in grass-thatched houses and the remainder in the metal-roofed houses. However, once inside the houses, proportions resting under the roof surfaces was generally lower in metal-roofed houses (*An. funestus*, 16.0% - 20.0%; *An. arabiensis*, 7.6% - 30.0%) than in grass-thatched houses (*An. funestus*, 32.5% - 55.2%; *An. arabiensis*, 43.1-49.8%). The proportions of mosquitoes resting on surfaces not typically sprayed were approximately one third in grass-thatched houses, and between one half and two third in metal-roofed houses. Full details including distribution of *Culex* mosquitoes are shown in Table 1.

Table 1: Numbers and percentages of mosquitoes of different species collected from different surfaces inside houses of different types

Species	Resting surfaces inside houses	Thatched roofs and mud walls	Thatched roofs and brick walls	Metal roofs and un-plastered brick walls	Metal roofs and plastered brick walls	Totals
		n (%)	n (%)	n (%)	n (%)	N (%)
<i>Anopheles funestus</i>	Walls	168 (17.9)	573 (24.5)	385 (37.1)	59 (27.4)	1185 (26.1)
	Roofs	519 (55.2)	762 (32.5)	166 (16.0)	43 (20.0)	1490 (32.9)
	Other surfaces	253 (26.9)	1008 (43.0)	486 (46.9)	113 (52.6)	1860 (41.0)
	<b>Total</b>	<b>940</b>	<b>2343</b>	<b>1037</b>	<b>215</b>	<b>4535</b>
<i>Anopheles arabiensis</i>	Walls	111 (21.0)	42 (12.7)	21 (26.6)	4 (10.0)	178 (18.2)
	Roofs	227 (43.1)	165 (49.8)	6 (7.6)	12 (30.0)	410 (42.0)
	Other surfaces	189 (35.9)	124 (37.5)	52 (65.8)	24 (60.0)	389 (39.8)
	<b>Total</b>	<b>527</b>	<b>331</b>	<b>79</b>	<b>40</b>	<b>977</b>
<i>Culex mosquitoes</i>	Walls	1089 (25.2)	700 (25.4)	683 (32.2)	929 (31.1)	3401 (27.9)
	Roofs	1926 (44.6)	1352 (49.0)	389 (18.3)	431 (14.4)	4098 (33.6)
	Other surfaces	1300 (30.1)	707 (25.6)	1051 (49.5)	1630 (54.5)	4688 (38.5)
	<b>Total</b>	<b>4315</b>	<b>2759</b>	<b>2123</b>	<b>2990</b>	<b>12 187</b>

Table 2 shows the extent to which mosquitoes preferred roofs and other internal house surfaces, compared to walls. Generally, the proportion of mosquitoes resting on non-sprayed surfaces (other surfaces) was always higher than proportions resting on walls regardless of house type. However, proportions resting on roofs was higher than on walls for grass-thatched houses, but lower for metal-roofed houses (Table 2).

When the data was examined for different house types, it became clear that wall surfaces, at best had only one third of mosquitoes resting. Depending on house construction materials, proportions of mosquitoes resting on roofs and other surfaces was often higher than on walls, except in metal-roofed houses, where walls tended to harbour more mosquitoes (Fig. 5 & 6). Data for *Culex* mosquitoes is shown in Tables 1 & 2, and Fig. 4 and 7. When the other surfaces were examined in detail, it was observed that significant proportions of mosquitoes on these surfaces were resting on bed-nets, floors, and on furniture, but also on hanging clothes. Full details are provided in Table 3.



Table 2: Summary statistics for comparison of the number of mosquitoes of different species collected from walls, roofs and other surfaces inside the different house types

House type	Resting surfaces	<i>Anopheles funestus</i>		<i>Anopheles arabiensis</i>		<i>Culex</i> mosquitoes	
		RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value
Thatched roofs and mud walls	Walls	1.00		1.00		1.00	
	Roofs	2.72 (2.11-3.50)	0.001	1.93 (1.33-2.79)	0.001	1.77 (1.50-2.08)	0.001
	Other surfaces	1.49 (1.14-1.94)	0.003	1.73 (1.20-2.51)	0.004	1.31 (1.11-1.54)	0.001
Thatched roofs and brick walls	Walls	1.00		1.00		1.00	
	Roofs	1.17 (0.94-1.45)	0.170	2.27 (2.27-2.28)	0.001	1.72 (1.37-2.16)	0.001
	Other surfaces	1.63 (1.31-2.02)	0.001	1.97 (1.96-1.97)	0.001	1.16 (0.92-1.47)	0.210
Metal roofs and un-plastered brick walls	Walls	1.00		1.00		1.00	
	Roofs	0.42 (0.32-0.57)	0.001	0.29 (0.12-0.71)	0.007	0.51 (0.37-0.69)	0.001
	Other surfaces	1.25 (0.96-1.63)	0.100	1.60 (0.93-2.78)	0.090	1.50 (1.15-1.97)	0.003
Metal roofs and plastered brick walls	Walls	1.00		-	-	1.00	
	Roofs	0.73 (0.50-1.07)	0.110	-	-	0.64 (0.48-0.83)	0.001
	Other surfaces	1.92 (1.41-2.59)	0.001	-	-	1.98 (1.56-2.52)	0.001

Very few *An. arabiensis* were caught in houses with metal roofs and plastered brick walls, it was not possible to fit GLMM model to *An. arabiensis* data

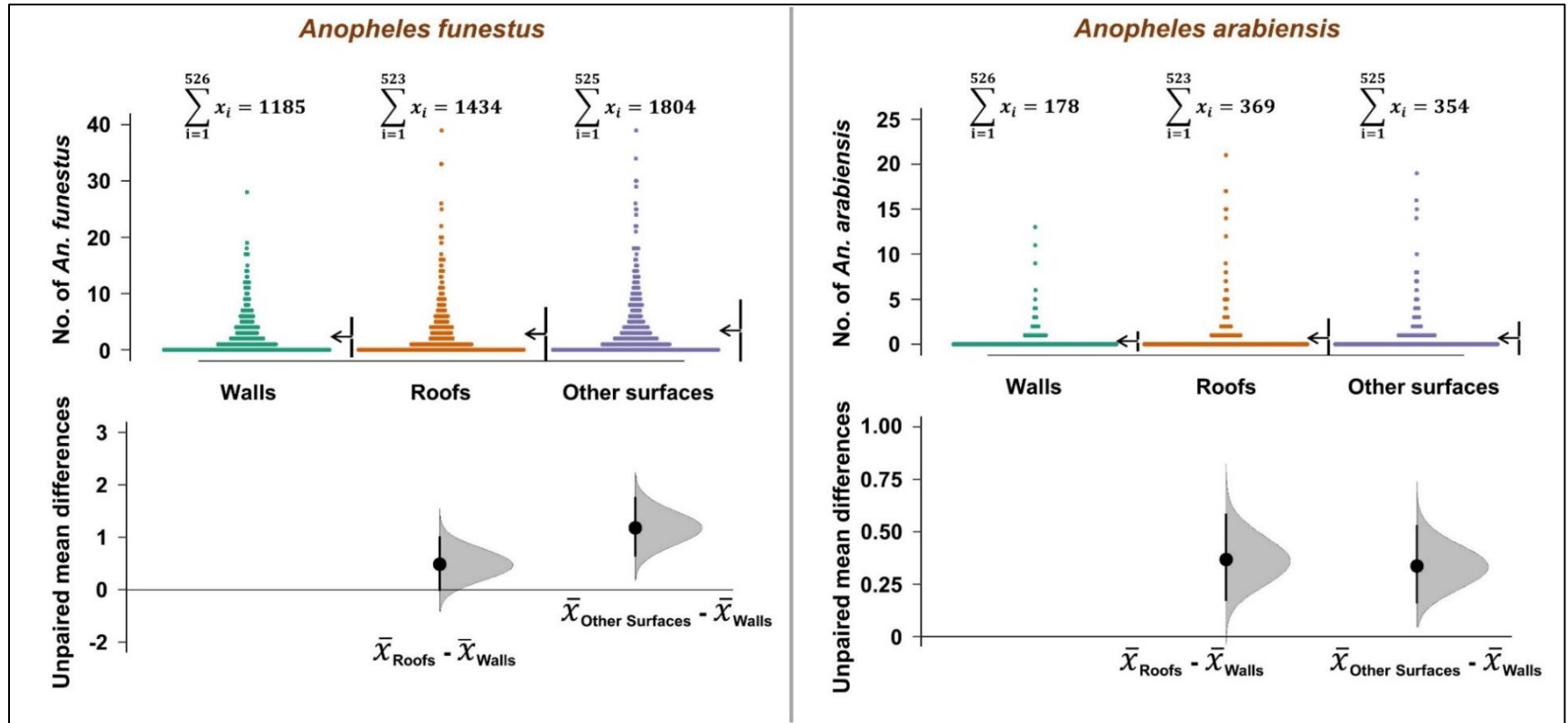


Figure 3: Overall nightly densities of malaria vectors *Anopheles funestus* and *Anopheles arabiensis*, from different resting surfaces inside the houses

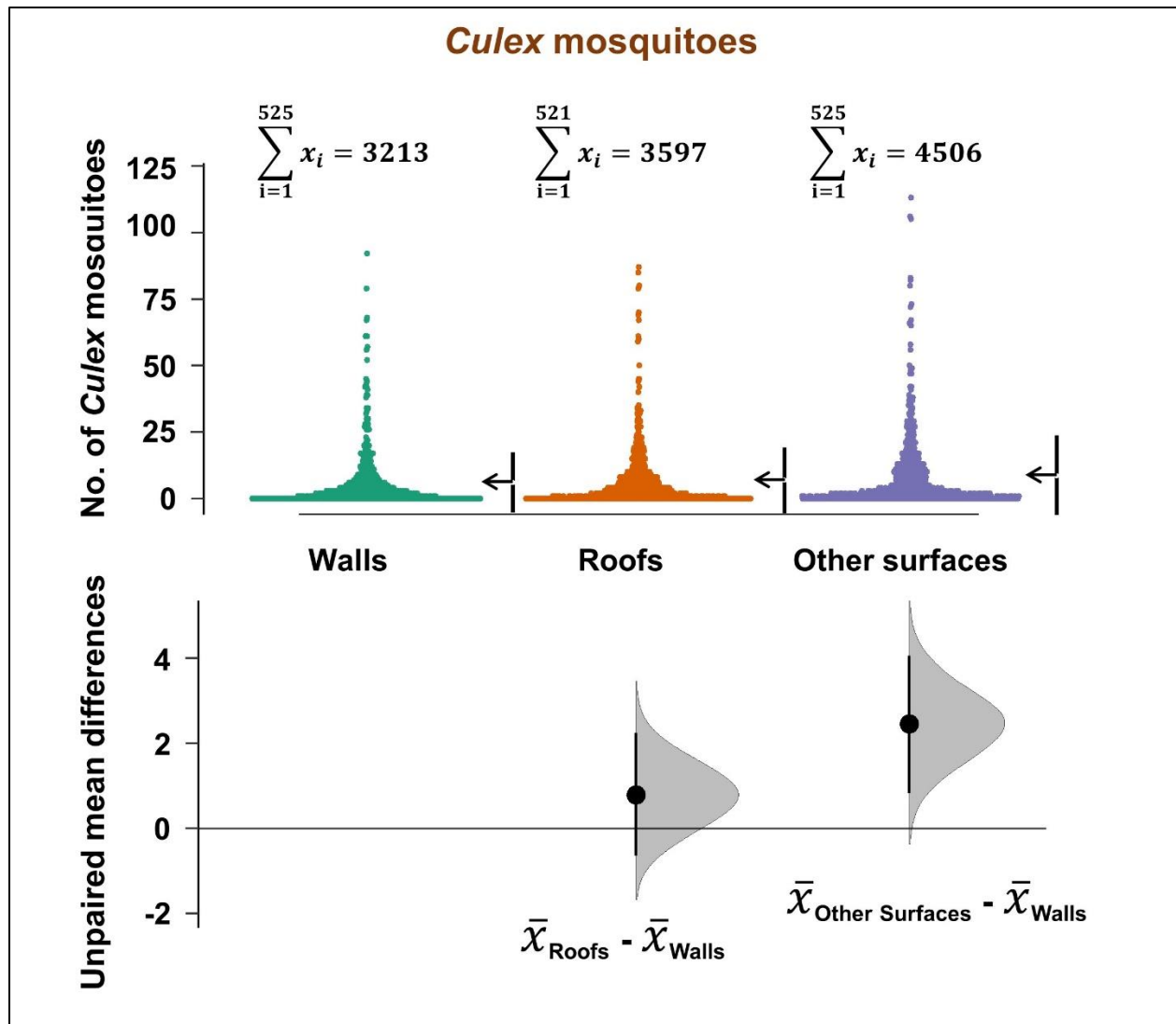


Figure 4: Overall densities of *Culex* mosquitoes, from different resting surfaces in houses

Table 3: Numbers and percentages of mosquitoes of different species collected from surfaces typically not targeted by IRS inside different houses types

Species	Resting surfaces inside houses	Thatched roofs and mud walls	Thatched roofs and brick walls	Metal roofs and un-plastered brick walls	Metal roofs and plastered brick walls	Totals
		n (%)	n (%)	n (%)	n (%)	N (%)
<i>Anopheles funestus</i>	Floor	129 (12.8)	29 (25.7)	125 (25.7)	48 (19.0)	331 (17.8)
	Furniture	186 (18.5)	18 (15.9)	80 (16.5)	87 (34.4)	371 (19.9)
	Bed-nets	587 (58.2)	25 (22.1)	79 (16.3)	59 (23.3)	750 (40.3)
	Clothes	74 (7.3)	31 (27.4)	134 (27.6)	32 (12.6)	271 (14.6)
	Utensils	32 (3.2)	10 (8.8)	68 (14.0)	27 (10.7)	137 (7.4)
	<b>Total</b>	<b>1008</b>	<b>113</b>	<b>486</b>	<b>253</b>	<b>1860</b>
<i>Anopheles arabiensis</i>	Floor	25 (20.2)	8 (33.3)	16 (30.8)	36 (19.0)	85 (21.9)
	Furniture	18 (14.5)	7 (29.2)	6 (11.5)	54 (28.6)	85 (21.9)
	Bed-nets	63 (50.8)	1 (4.2)	15 (28.8)	24 (12.7)	103 (26.5)
	Clothes	9 (7.3)	3 (12.5)	9 (17.3)	41 (21.7)	62 (15.9)
	Utensils	9 (7.3)	5 (20.8)	6 (11.5)	34 (18.0)	54 (13.9)
	<b>Total</b>	<b>124</b>	<b>24</b>	<b>52</b>	<b>189</b>	<b>389</b>
<i>Culex mosquitoes</i>	Floor	209 (29.6)	458 (28.1)	275 (26.2)	261 (20.1)	1203 (25.7)
	Furniture	189 (26.7)	470 (28.8)	191 (18.2)	461 (35.5)	1311 (28.0)
	Bed-nets	100 (14.1)	123 (7.5)	236 (22.5)	100 (7.7)	559 (11.9)
	Clothes	125 (17.7)	368 (22.6)	175 (16.7)	236 (18.2)	904 (19.3)
	Utensils	84 (11.9)	211 (12.9)	174 (16.6)	242 (18.6)	711 (15.2)
	<b>Total</b>	<b>707</b>	<b>1630</b>	<b>1051</b>	<b>1300</b>	<b>4688</b>

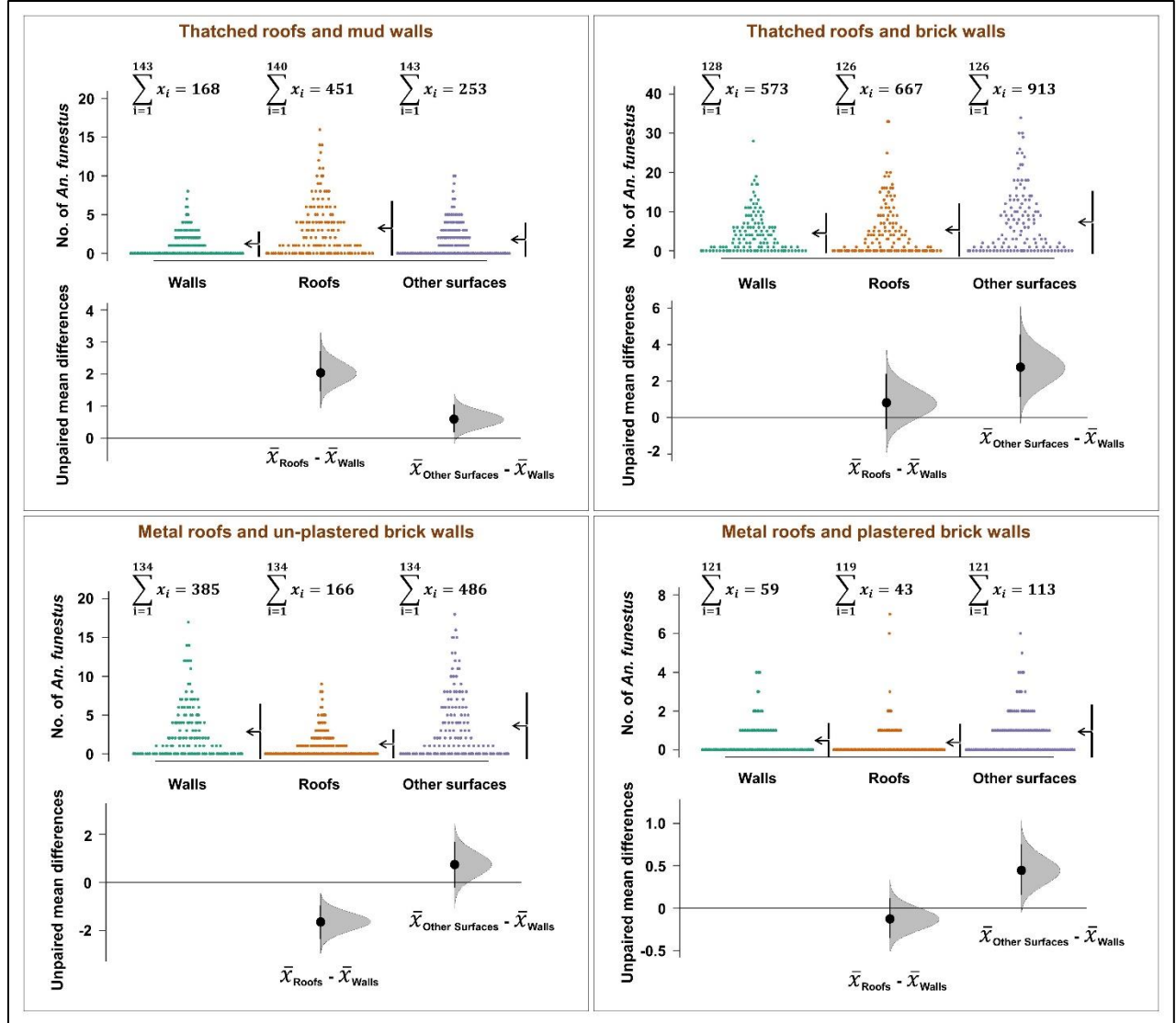


Figure 5: Comparison of *Anopheles funestus* densities on different resting surfaces in different house types

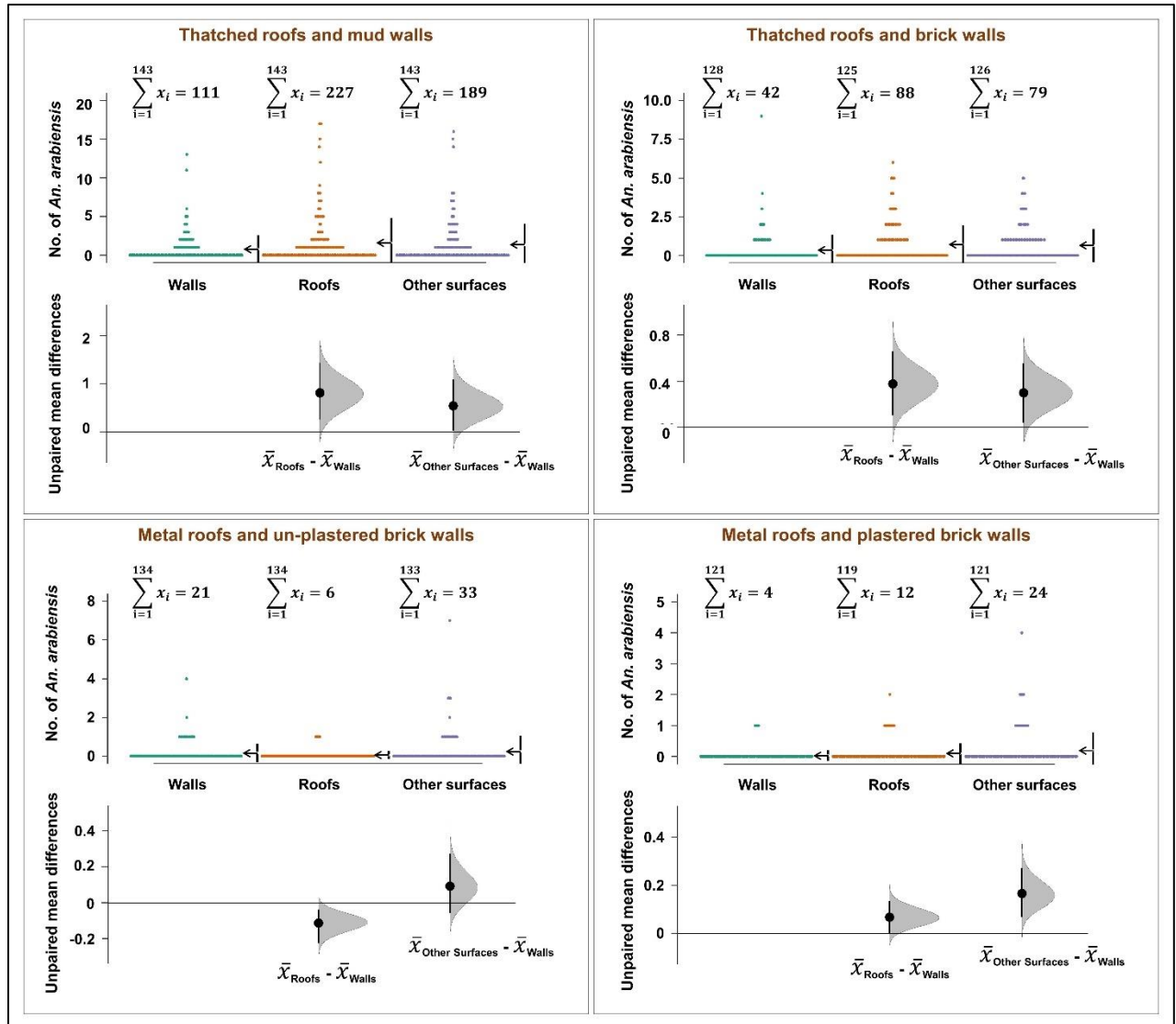


Figure 6: Comparison of *Anopheles arabiensis* densities on different resting surfaces in different house types

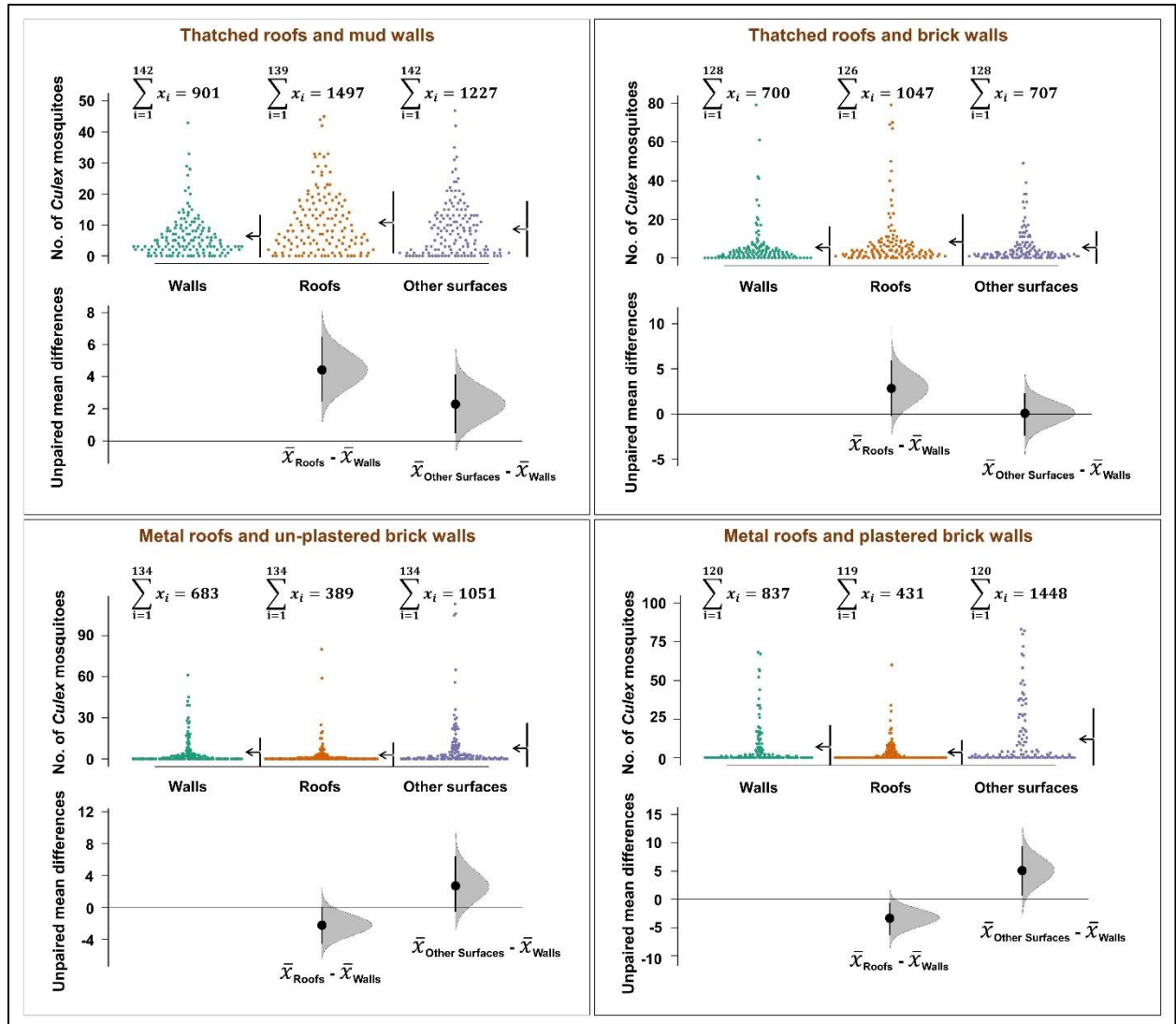


Figure 7: Comparison of densities of *Culex* mosquitoes, from different resting surfaces in different house types

#### **4.1.3. Effects of household variables on preferences of mosquitoes for different resting surfaces inside houses.**

Associations between household risk factors and proportions of mosquitoes in different resting surfaces are summarized in Table 4. Generally, compared to metal-roofed houses, grass-thatched houses had more mosquitoes of all taxa, and on all surfaces. In most cases, the number of mosquitoes in grass-thatched houses was more than double that in metal-roofed houses. Compared to brick walled houses, the mud-walled houses had less mosquitoes of all taxa, on any surface assessed. These differences varied but were significantly four times less for *An. funestus* ( $p=0.01$ ) (Table 4). Leaving walls un-plastered was also associated with greater *Anopheles* density on the walls, significantly more so with *An. funestus*. This effect was less evident when considering mosquitoes collected from roofs or other surfaces. Similarly, leaving the eave spaces open was associated with higher vector densities on the walls and other surfaces, but not on roofs. Finally, there were more mosquitoes on walls below one metre. Full details of this analysis are provided in Table 4.



Table 4: Relationship between of household risk factors and indoor temperatures on mosquito resting preference on different surfaces

Variable	Categories	<i>Anopheles funestus</i>		<i>Anopheles arabiensis</i>		<i>Culex</i> mosquitoes	
		RR (95% CI)	p value	RR (95% CI)	p value	RR (95% CI)	p value
Number of mosquitoes caught resting on walls							
Roof type	Iron sheets	1.00		1.00		1.00	
	Grass thatch	2.20 (0.87-5.56)	0.090	1.93 (0.46-8.10)	0.400	1.04 (0.43-2.46)	0.940
Wall type	Brick	1.00		1.00		1.00	
	Mud	0.17 (0.07-0.41)	0.001	0.33 (0.09-1.24)	0.100	0.97 (0.42-2.26)	0.950
Interior walls	Plastered	1.00		1.00		1.00	
	Un-plastered	3.66 (1.34-10.02)	0.010	1.65 (0.22-12.25)	0.620	0.96 (0.38-2.48)	0.940
Eave space	Closed	1.00		1.00		1.00	
	Open	0.38 (0.13-1.13)	0.080	1.68 (0.23-12.26)	0.610	1.04 (0.40-2.74)	0.940
Increasing No. rooms		1.51 (1.08-2.11)	0.020	2.48 (1.38-4.47)	0.002	1.16 (0.86-1.58)	0.330
Increasing wall height		0.38 (0.13-1.11)	0.080	0.12 (0.02-0.79)	0.030	1.05 (0.42-2.65)	0.910
Increasing max. temp.		0.97 (0.93-1.02)	0.220	1.16 (1.07-1.25)	0.001	1.02 (0.97-1.07)	0.410
Number of mosquitoes caught resting on the underside of roofs							
Roof types	Iron sheet	1.00		1.00		1.00	
	Grass thatch	6.07 (1.78-20.70)	0.004	92.16 (9.90-857.94)	0.001	3.96 (1.51-10.36)	0.005
Wall type	Brick	1.00		1.00		1.00	
	Mud	0.27 (0.09-0.77)	0.010	0.39 (0.09-1.68)	0.210	0.80 (0.32-2.01)	0.630
Interior walls	Plastered	1.00		1.00		1.00	
	Un-plastered	1.55 (0.44-5.46)	0.500	0.29 (0.02-4.74)	0.390	0.74 (0.26-2.12)	0.570
Eave space	Closed	1.00		1.00		1.00	
	Open	0.25 (0.06-0.98)	0.046	0.91 (0.06-14.53)	0.910	0.53 (0.18-1.54)	0.240
Increasing No. rooms		1.48 (0.97-2.26)	0.070	2.49 (1.25-4.96)	0.010	1.44 (1.03-2.00)	0.030
Increasing wall height		0.25 (0.06-0.95)	0.040	0.55 (0.06-4.71)	0.570	0.87 (0.31-2.41)	0.790
Increasing max. temp.		1.02 (0.97-1.07)	0.410	1.13 (1.06-1.20)	0.001	0.98 (0.93-1.02)	0.300
Number of mosquitoes caught resting on other surfaces inside the houses							
Roof types	Iron sheet	1.00		1.00		1.00	
	Grass thatch	2.12 (0.85-5.31)	0.110	3.75 (0.88-16.03)	0.070	1.66 (0.68-4.02)	0.260
Wall type	Brick	1.00		1.00		1.00	
	Mud	0.22 (0.09-0.55)	0.001	0.59 (0.13-2.78)	0.510	0.92 (0.38-2.21)	0.840
Interior walls	Plastered	1.00		1.00		1.00	
	Un-plastered	0.92 (0.33-2.54)	0.870	2.77 (0.44-17.51)	0.280	0.81 (0.30-2.13)	0.660
Eave space	Closed	1.00		1.00		1.00	
	Open	2.91 (1.00-8.46)	0.049	1.95 (0.28-13.71)	0.500	1.23 (0.46-3.33)	0.680
Increasing No. rooms		1.16 (0.84-1.60)	0.370	1.61 (0.90-2.88)	0.110	1.25 (0.91-1.71)	0.160
Increasing wall height		0.84 (0.29-2.41)	0.750	2.68 (0.46-15.65)	0.270	3.18 (1.21-8.33)	0.020
Increasing max. temp.		0.93 (0.90-0.97)	0.001	1.12 (1.06-1.19)	0.001	1.03 (0.98-1.08)	0.280

#### **4.1.4. *Anopheles* sibling species and *Plasmodium* infections**

A subsample of 191 *An. gambiae* s.l and 623 *An. funestus* s.l were assayed for identification of sibling species, and presence of infectious stages of *P. falciparum*, i.e. sporozoites in the salivary glands. In the *An. gambiae* s.l samples, there was an overall PCR amplification of 93.2% (n = 178), of which 100% were *An. arabiensis*, and none had sporozoite infections. For *An. funestus* s.l, PCR amplification was 89.1% (n = 555), of which 93.1% were *An. funestus sensu stricto* (s.s.) (n = 517), and 6.8% were *Anopheles rivulorum* (n = 38). None of the *An. rivulorum*, nor the un-amplified samples had sporozoites infections, but four of the *An. funestus* s.s. mosquitoes were sporozoites positive (0.8%).

#### **4.1.5. Mosquito blood meal sources and parity statuses**

Based on the blood-meal ELISA assays done on 45 blood-fed *An. arabiensis*, more than half had human blood (55.56%; n = 25). The rest had blood from cattle (20%; n = 9), dogs (15.6%; n = 7), chickens (2.2%; n = 1) as well as mixed blood from dogs and cattle (4.4%; n = 2) and from humans and dogs (2.2%; n = 1). For *An. funestus* s.s., 224 blood-fed females were tested, the majority of which had obtained blood from humans (90.6%; n = 203). The rest of the *An. funestus* had blood from chicken (2.2%; n = 5), cattle (1.8%; n = 4), dog (0.9%; n = 2), mixtures of human and cattle blood (2.7%; n = 6) or human and chicken blood (1.8%; n = 4). Lastly, for *An. rivulorum*, only seven samples were tested, six of which had human blood in their guts (85.7%), the other having fed on cattle (14.3%).

Of 67 *An. arabiensis* dissected, 53.7% (n=36) were parous and 46.3% (n=31) were nulliparous. While of 160 *An. funestus* dissected, only 36.9% (n=59) were parous and the rest were nulliparous.

## 4.2. Discussion

This research investigated the resting behaviours of malaria mosquitoes inside typical house types in rural south-eastern Tanzanian villages where *An. arabiensis* and *An. funestus* are the main vectors, the latter contributing more than 80% of all cases (Kaindoa *et al.*, 2017). The main finding was that consistently less than one third of mosquitoes that enter houses typically rest on walls, which are the main target for IRS campaigns. In fact, significant proportions regularly rest on surfaces other than walls or roofs (which are also sometime sprayed). These other surfaces include household items such as furniture, utensils, clothing and also on floors, places that are rarely sprayed. As historically observed (Smith, 1962b, 1962a), this current study determined that malaria vectors do not rest only on walls, where they can be targeted with IRS. Instead, all surfaces inside houses are potential resting site for mosquitoes. Majority of *An. funestus* and *An. arabiensis* rest on surfaces other than walls, such as on the underside of roofs, bed-nets, floors, furniture, utensils and clothes. However, variations were observed between vector species and house designs.

Indoor residual spraying and long-lasting insecticide-treated nets, despite having been tremendously impactful (Bhatt *et al.*, 2015), are now perceived as inadequate for the goal of malaria elimination (Rabinovich *et al.*, 2017; The malERA Consultative Group on Vector Control, 2011; The malERA Refresh Consultative Panel on Tools for Malaria Elimination, 2017), partly due to the rise of insecticide resistance (Hemingway *et al.*, 2016; Ranson & Lissenden, 2016) and changes in mosquito biting behaviours (Finda *et al.*, 2019; Monroe *et al.*, 2019; Sherrard-Smith *et al.*, 2019). These challenges may result from, and can be compounded by extensive and improper implementation of the insecticide based strategies (Killeen & Chitnis, 2014; Protopopoff *et al.*, 2008; Stump *et al.*, 2004). For example, incomplete coverage of all mosquito resting surfaces with IRS inside houses could lead to lower coverage of indoor surfaces with insecticides, sub-optimal dosing of the mosquitoes and hence reduced communal impact of the interventions. Therefore, to attain malaria elimination targets, current interventions need improvements to maximize effectiveness. This requires extensive understanding of mosquito behaviours inside houses, and how these mosquitoes would respond to indoor interventions, notably IRS and ITNs.

The composition of indoor resting mosquitoes observed in this study was of fairly different physiological ages and few infectious *Anopheles*. Also, bloodmeal sources suggests that even outdoor biting mosquitoes rested indoor. This study therefore provides evidence that expanding target surfaces inside houses when spraying insecticides would increase impact of IRS on mosquito populations. Where this is not possible, a behaviour change communication program can be implemented to sensitize and educate people on dangers of mosquitoes resting indoors on surfaces such as hanged clothes. IRS campaigns usually involve removal of household items before spraying is conducted (WHO, 2015a). However, once these items are returned to the houses, they form important resting surfaces free of insecticides. Since the study involved multiple collections at different times of day and night, the observed resting patterns are likely the natural patterns. It is however unclear whether there are any frequent movements of mosquitoes between resting surfaces, and how such movements may influence overall impact of IRS.

More importantly, these findings highlight specific gaps and limitations of IRS, and the need for more comprehensive interventions such as house improvement. As an example, house screening would not be affected by mosquito resting behaviours but would instead reduce overall densities in the homes. Another way would be to expand, as much as possible, the IRS targeted surfaces to include undersides of roofs and other sprayable surfaces (such as underneath beds, tables and other furniture) to have increased impact on the mosquitoes. Thirdly, coupling IRS with strategies to minimize mosquito resting on non-sprayable surfaces might also enhance impact. Such strategies may include, but are not limited to proper storage of household items, e.g. by placing these items inside enclosures such as cupboards. This could reduce potential surfaces for mosquito to rest, which may maximize mosquito contacts with treated surfaces. Without considering surfaces other than walls, our current efforts, targeting mosquito vectors with IRS might limit the impact of IRS on elimination and control outcomes. However, one would argue that proportion of mosquitoes resting on surfaces other than walls does not mean that these mosquitoes would never come into contact with walls or wouldn't be killed by IRS.

Indoor residual spraying remains one of the mainstays of malaria control in Africa, and is widely popular despite high costs. It is currently promoted in Africa mostly through the US Presidents Malaria Initiative (U.S. President's Malaria Initiative, 2019b) and national programs often alongside LLINs, but was historically the most dominant tool in Africa and elsewhere starting

from the Global Malaria Eradication period (Kouznetsov, 1977; Nájera *et al.*, 2011). It has indeed been associated with major reductions in malaria cases in the southern Africa region in past decades (Mabaso *et al.*, 2004; Sharp *et al.*, 2007), and remains an important component of their malaria control arsenal. The spraying procedures are generally standardized to achieve scale and reduce costs (WHO, 2015a), and generally target walls and ceilings occasionally where these exist. As a result, the spraying operations may not adequately capture the full-spectrum of resting spaces used by malaria vectors or others.

The findings of this current study are in line with previous studies on resting preference of *Anopheles* mosquitoes inside houses (Smith, 1962a). However, this study extended the mosquito collections to cover more potential sites inside human inhabited dwellings, and also examined differences between different house types. It also described relationships between house designs and microclimate, with resting preferences of the *An. funestus*, *An. arabiensis*, and *Culex* mosquitoes. For example, grass thatched roofs were associated with higher proportions of *An. funestus* on roofs. When houses had open eaves, proportion of *An. funestus* increased on other surfaces, but increase in indoor maximum temperature was associated with decrease in proportion of *An. funestus* on other surfaces.

Insecticide resistance has led to a change of insecticides used in IRS to non-pyrethroid insecticides such as pirimiphos-methyl and neonicotinoids (Hemingway *et al.*, 2016; WHO, 2018b). However, recently a countrywide survey in Tanzania detected resistance against pirimiphos-methyl in several sites within the country (Kisiza *et al.*, 2017). It was observed that out 20 sites, three sites had *An. gambiae* s.l population resistant to pirimiphos-methyl and elevated levels of glutathione S-transferases, nonspecific esterases, acetyl- cholinesterase and mixed function oxidases enzymes (Kisiza *et al.*, 2017). Suspected cause of this resistance was common use of insecticides classes in agriculture as that in malaria vector control (Kisiza *et al.*, 2017). Results of this study indicates that current IRS practices clearly miss several surfaces where mosquitoes rest, a situation, which could exacerbate the challenge of insecticide resistance and further compromise IRS. As mentioned earlier in this paper, understanding the resting behaviours of malaria vectors is crucial, if at all IRS is going to be widely used in malaria endemic countries including Tanzania. The gaps identified in this study can be compounded by insecticide resistance, and therefore need urgent attention to ensured effectiveness.

Though mostly successful, this study also had a few limitations. First, most collections of mosquitoes were done in the morning, when people were active participating in household chores. This might have influenced the choice of mosquitoes on resting surfaces during the day. Collections during the day might also have underestimated mosquitoes resting on surfaces such as floors and utensils. Second, the type and number of possessions inside houses are related to house types, since both are linked to wealthy/income. Mud houses are unlikely to have bigger furniture and rarely items inside these houses are properly arranged. It is likely that resting patterns of mosquitoes between individual house type was influenced by type and number of surfaces inside houses. Thus, influencing observed differences in resting preference among house types involved. Unfortunately, this phenomenon was not assessed in this study. Third, this study was conducted in villages which are not protected with IRS. However, mosquitoes have been shown in multiple studies to change their behaviours with interventions. Therefore, it is important that future studies should be carried-out to assess indoor resting preference of mosquitoes in houses protected with IRS.

## **CHAPTER FIVE**

### **CONCLUSION AND RECOMMENDATIONS**

#### **5.1. Conclusion**

This study has demonstrated that while IRS typically uses contact insecticides against adult mosquitoes on walls, and occasionally roofs and ceilings, significant proportions of malaria vectors rest on other surfaces not usually sprayed during IRS campaigns. The study also demonstrated that the spraying gaps are influenced by house designs. For example, in grass-thatched houses, up to one third of mosquitoes consistently rest on surfaces other than walls or roofs, are therefore not effectively controlled by contact insecticides. These gaps can reach two-thirds of mosquitoes in metal-roofed houses.

Given that roofs are often ignored during IRS, it is likely that there are even wider gaps in IRS operations. Similarly, any attempts by IRS implementers to reduce the surface area sprayed (e.g. to reduce costs and increase coverage), should be analyzed based on local evidence of mosquito-resting preferences. Expanding IRS targeted surfaces inside houses can also be impactful. However, given the costs of IRS and logistical challenges associated with spraying non-standard surfaces, this approach in resource limited settings may not be sustainable. It remains unclear how the observed mosquito habits could impact overall effectiveness of IRS.

Most importantly therefore, there is need to incorporate locally-obtained data on mosquito resting behaviours to maximize potential of IRS. Besides, other interventions such as improved housing should be prioritized to more comprehensively tackle indoor-biting and indoor-resting mosquitoes.

#### **5.2. Recommendations**

Based on the observation made in this study that, only less than one-third of mosquito were found rest on walls and significant proportions of vectors rest on surfaces not usually sprayed. In houses with thatch roofs mosquitoes resting on surfaces that are not sprayed can exceed one-third and in houses with metal roofs can be more than two-third. Also, with observation that house structure significantly influences indoor mosquito density and resting preferences, recommendations from this study include the following:

- (i) Indoor residual spraying should rely on house designs for targeting IRS activities. House design significantly influence indoor mosquito density and resting preferences. Therefore, IRS activities should rely on house design and focus its activities in houses that tend to have higher densities of mosquitoes, for example e.g. by focusing on thatch-roofed houses and houses without plastered walls.
- (ii) For houses with thatched roofs, target surfaces for spraying residual insecticide should be expanded to include roofs, especially in occasions when roofs are not considered for spraying. In houses with thatch roofs, roofs were observed to harbor large proportion of mosquitoes. Therefore, by targeting this surface majority of mosquitoes in these houses can be impacted with IRS, thereby increasing the impact of IRS.
- (iii) Implement behaviour change communication programs to raise awareness of indoor mosquito resting places. These programs can be implemented to sensitize and educate people on dangers of mosquitoes resting indoors on surfaces such as hanged clothes.
- (iv) Coupling IRS with other strategies to minimize mosquito resting on non-sprayable surfaces. As an example, by minimizing the number of resting surfaces that are not targeted by indoor residual spraying inside houses. This could reduce potential surfaces for mosquito to rest, which may maximize mosquito contacts with treated surfaces.
- (v) Consider other options that target all mosquitoes. For example, option such as:
  - a) House improvement will reduce overall mosquito densities inside houses.
  - b) Insecticide-treated eave ribbons which target mosquitoes before they enter houses, thus increasing impact.



## REFERENCES

- Alba, S., Nathan, R., Schulze, A., Mshinda, H., & Lengeler, C. (2014). Child mortality patterns in rural Tanzania: an observational study on the impact of malaria control interventions. *International Journal of Epidemiology*, 43(1), 204–215. <https://doi.org/doi:10.1093/ije/dyt231>.
- Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Bayoh, M. N., Mathias, D. K., Odiere, M. R., Mutuku, F. M., Kamau, L., Gimnig, J. E., Vulule, J. M., Hawley, W. A., Hamel, M. J., & Walker, E. D. (2010). *Anopheles gambiae*: Historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malaria Journal*, 9(1). <https://doi.org/10.1186/1475-2875-9-62>.
- Beier, J. C., Perkins, P. V., Wirtz, R. A., Koros, J., Diggs, D., Gargan, T. P., & Koech, D. K. (1988). Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology*, 25(1), 9–16. <https://doi.org/10.1093/jmedent/25.1.9>.
- Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K. E., Moyes, C. L., Henry, A., Penny, M. A., Smith, T. A., Bennett, A., Yukich, J., Eisele, T. P., Eckhoff, P. A., Wenger, E. A., Brie, O., Griffin, J. T., Fergus, C. A., ... Gething, P. W. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature*, 526(7572), 207–211. <https://doi.org/10.1038/nature15535>.
- Bhattarai, A., Ali, A. S., Kachur, S. P., Mårtensson, A., Abbas, A. K., Khatib, R., Al-mafazy, A. W., Ramsan, M., Rotllant, G., Gerstenmaier, J. F., Molteni, F., Abdulla, S., Montgomery, S. M., Kaneko, A., & Björkman, A. (2007). Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS Medicine*, 4(11), 1784–1790. <https://doi.org/10.1371/journal.pmed.0040309>.

- Bolker, B. (2007). *Ecological Models and Data in R*. Princeton University Press.
- Coetzee, M., Hunt, R. H., Wilkerson, R., Torre, A. Della, Coulibaly, M. B., & Besansky, N. J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, 3619(3), 246–274. <https://doi.org/10.11646/zootaxa.3619.3.2>
- De Meillon, B. (1934). Entomological Studies-Observations on *Anopheles funestus* and *Anopheles gambiae* in the Transvaal. *Publication of the South African Institute for Medical Research*, 32, 199–248.
- Detinova, T. S. (1962). *Age-grouping methods in diptere of medical importance*. World Health Organization.
- Durnez, L., Van Bortel, W., Denis, L., Roelants, P., Veracx, A., Trung, H. D., Sochantha, T., & Coosemans, M. (2011). False positive circumsporozoite protein ELISA: A challenge for the estimation of the entomological inoculation rate of malaria and for vector incrimination. *Malaria Journal*, 10(1), 195. <https://doi.org/10.1186/1475-2875-10-195>.
- Finda, M. F., Moshi, I. R., Monroe, A., Limwagu, A. J., Nyoni, P., Swai, J. K., Ngowo, H. S., Minja, E. G., Toe, L. P., Kaindoa, W., Coetzee, M., Manderson, L., & Okumu, F. O. (2019). Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. *PLoS One*, 14(6), e0217414. <https://doi.org/10.1371/journal.pone.0217414>.
- Gibbins, E. G. (1933). The domestic *Anopheles* mosquitoes of Uganda. *Annals of Tropical Medicine and Parasitology*, 27(1), 15–25. <https://doi.org/10.1080/00034983.1933.11684736>.
- Gillies, M. T., & Coetzee, M. (1987). *A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region)*. South African Medical Research Institute.
- Gleave, K., Lissenden, N., Richardson, M., Choi, L., & Ranson, H. (2018). Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. *Cochrane Database of Systematic Reviews*, 11, CD012776. <https://doi.org/10.1002/14651858.CD012776.pub2>.

- Haddow, A. J. (1942). The mosquito fauna and climate of native huts at kisumu, Kenya. *Bulletin of Entomological Research*, 33(2), 91–142. <https://doi.org/10.1017/S0007485300026389>.
- Hemingway, J., Ranson, H., Magill, A., Kolaczinski, J., Fornadel, C., Gimnig, J., Coetzee, M., Simard, F., Roch, D. K., Hinzoumbe, C. K., Pickett, J., Schellenberg, D., Gething, P., Hoppé, M., & Hamon, N. (2016). Averting a malaria disaster: Will insecticide resistance derail malaria control? *The Lancet*, 387(10029), 1785–1788. [https://doi.org/10.1016/S0140-6736\(15\)00417-1](https://doi.org/10.1016/S0140-6736(15)00417-1).
- Ho, J., Tumkaya, T., Aryal, S., Choi, H., & Claridge-Chang, A. (2018). Moving beyond P values: Everyday data analysis with estimation plots. *Nature Methods*, 16(7), 565–566. <https://doi.org/10.1101/377978>.
- Kaindoa, E. W., Matowo, N. S., Ngowo, H. S., Mkandawile, G., Mmbando, A., Finda, M., & Okumu, F. O. (2017). Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south–eastern Tanzania. *PLoS ONE*, 12(5), e0177807. <https://doi.org/10.1371/journal.pone.0177807>.
- Killeen, G. F., & Chitnis, N. (2014). Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis. *Malaria Journal*, 13(1), 97. <https://doi.org/10.1186/1475-2875-13-97>.
- Kirby, M. J., Ameh, D., Green, C., Jawara, M., Milligan, P. J., Snell, P. C., Conway, D. J., & Lindsay, S. W. (2013). Efficacy of two different house screening interventions against exposure to malaria and anaemia in children in The Gambia: a randomized controlled trial. *The Lancet*, 374(9694), 998–1009. [https://doi.org/10.1016/S0140-6736\(09\)60871-0](https://doi.org/10.1016/S0140-6736(09)60871-0).Efficacy.
- Kisizza, W. N., Nkya, T. E., Kabula, B., Overgaard, H. J., Massue, D. J., Mageni, Z., Greer, G., Kaspar, N., Mohamed, M., Reithinger, R., Moore, S., Lorenz, L. M., & Magesa, S. (2017). Multiple insecticide resistance in *Anopheles gambiae* from Tanzania: A major concern for malaria vector control. *Malaria Journal*, 16(1), 439. <https://doi.org/10.1186/s12936-017-2087-2>.

- Koekemoer, L. L., Kamau, L., Hunt, R. H., & Coetzee, M. (2002). A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *The American Journal of Tropical Medicine and Hygiene*, 66(6), 804–811. <https://doi.org/10.4269/ajtmh.2002.66.804>.
- Kouznetsov, R. L. (1977). Malaria control by application of indoor spraying of residual insecticides in tropical Africa and its impact on community health. *Tropical Doctor*, 7(2), 81–91. <https://doi.org/10.1177/004947557700700216>.
- Lindsay, S. W., Jawara, M., Mwesigwa, J., Achan, J., Bayoh, N., Bradley, J., Kandeh, B., Kirby, M. J., Knudsen, J., Macdonald, M., Pinder, M., Tusting, L. S., Weiss, D. J., Wilson, A. L., & Alessandro, U. D. (2019). Reduced mosquito survival in metal-roof houses may contribute to a decline in malaria transmission in sub-Saharan Africa. *Scientific Reports*, 9(1), 7770. <https://doi.org/10.1038/s41598-019-43816-0>.
- Lindsay, S. W., Jawara, M., Paine, K., Pinder, M., Walraven, G. E. L., & Emerson, P. M. (2003). Changes in house design reduce exposure to malaria mosquitoes. *Tropical Medicine and International Health*, 8(6), 512–517. <https://doi.org/10.1046/j.1365-3156.2003.01059.x>.
- Lwetoijera, D. W., Harris, C., Kiware, S. S., Dongus, S., Devine, G. J., McCall, P. J., & Majambere, S. (2014). Increasing role of *Anopheles funestus* and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. *Malaria Journal*, 13(1), 331. <https://doi.org/10.1186/1475-2875-13-331>.
- Mabaso, M. L. H., Sharp, B., & Lengeler, C. (2004). Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. *Tropical Medicine and International Health*, 9(8), 846–856.
- Maia, M. F., Robinson, A., John, A., Mgando, J., Simfukwe, E., & Moore, S. J. (2011). Comparison of the CDC Backpack aspirator and the Prokopack aspirator for sampling indoor- and outdoor-resting mosquitoes in southern Tanzania. *Parasites & Vectors*, 4(1), 124. <https://doi.org/10.1186/1756-3305-4-124>.
- Mashauri, F. M., Kinung'hi, S. M., Kaatano, G. M., Magesa, S. M., Kishamawe, C., Mwanga, J.

- R., Nnko, S. E., Malima, R. C., Mero, C. N., & Mboera, L. E. G. (2013). Impact of Indoor Residual Spraying of Lambda-Cyhalothrin on Malaria Prevalence and Anemia in an Epidemic-Prone District of Muleba, North-Western Tanzania. *The American Journal of Tropical Medicine and Hygiene*, 88(5), 841–849. <https://doi.org/10.4269/ajtmh.12-0412>.
- Mathis, W., ST. Cloud, A., Eyraud, M., Miller, S., & Hamon, J. (1963). Initial Field Studies in Upper Volta with Dichlorvos Residual Fumigant as a Malaria Eradication Technique. *Bulletin of the World Health Organization*, 29(1961), 237–241.
- Matowo, N. S., Abbasi, S., Munhenga, G., Tanner, M., Mapua, S. A., Oullo, D., Koekemoer, L. L., Kaingoa, E., Ngowo, H. S., Coetzee, M., Utzinger, J., & Okumu, F. O. (2019). Fine - scale spatial and temporal variations in insecticide resistance in *Culex pipiens* complex mosquitoes in rural south - eastern Tanzania. *Parasites & Vectors*, 12(1), 413. <https://doi.org/10.1186/s13071-019-3676-4>.
- Moiroux, N., Gomez, M. B., Pennetier, C., Elanga, E., Djènontin, A., Chandre, F., Djègbé, I., Guis, H., & Corbel, V. (2012). Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *Journal of Infectious Diseases*, 206(10), 1622–1629. <https://doi.org/10.1093/infdis/jis565>.
- Monroe, A., Moore, S., Koenker, H., Lynch, M., & Ricotta, E. (2019). Measuring and characterizing night time human behaviour as it relates to residual malaria transmission in sub - Saharan Africa: a review of the published literature. *Malaria Journal*, 18(1), 6. <https://doi.org/10.1186/s12936-019-2638-9>.
- Nagpal, B. N., Srivastava, A., & Dash, A. P. (2012). Resting behaviour of *Anopheles stephensi* type form to assess its amenability to control malaria through indoor residual spray. *Journal of Vector Borne Diseases*, 49(3), 175–180.
- Nájera, J. A., González-Silva, M., & Alonso, P. L. (2011). Some lessons for the future from the global malaria eradication programme (1955-1969). *PLoS Medicine*, 8(1), e1000412. <https://doi.org/10.1371/journal.pmed.1000412>.
- National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS),

- Ministry of Health, Community Development, Gender, Elderly and Children (MOHCDGEC) [Tanzania Mainland], Ministry of Health (MOH) [Zanzibar], & ICF. (2018). *Tanzania Malaria Indicator Survey*. MoHCDGEC, MoH, NBS, OCGS, ICF.
- National Malaria Control Programme. (2014). *National Malaria Strategic Plan 2014–2020* (Issue January). Ministry of Health and Social Welfare.
- Nkya, T. E., Poupardin, R., Laporte, F., Akhouayri, I., Mosha, F., Magesa, S., Kisinza, W., & David, J. P. (2014). Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: a multigenerational study in controlled conditions. *Parasites & Vectors*, 7(1), 480. <https://doi.org/10.1186/s13071-014-0480-z>.
- Ogata, K., Ikeda, T., Umino, T., & Bocanegra, R. Z. (1992). Observations of biting and resting behavior of *Anopheles albimanus* in Guatemala. *Japanese Journal of Sanitary Zoology*, 43(1), 47–57.
- Okech, B. A., Gouagna, L. C., Knols, B. G. J., Kabiru, E. W., Killeen, G. F., Beier, J. C., Yan, G., & Githure, J. I. (2004). Influence of indoor microclimate and diet on survival of *Anopheles gambiae* s.s. (Diptera: Culicidae) in village house conditions in western Kenya. *International Journal of Tropical Insect Science*, 24(3), 207–212. <https://doi.org/10.1079/IJT200427>.
- Pates, H., & Curtis, C. (2005). Mosquito behavior and vector control. *Annual Review of Entomology*, 50(1), 53–70. <https://doi.org/10.1146/annurev.ento.50.071803.130439>.
- Pringle, G. (1967). Malaria in the pare area of Tanzania III The course of malaria transmission since the suspension of an experimental programme of residual insecticide spraying. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 61(1), 69–79. [https://doi.org/10.1016/0035-9203\(46\)90076-4](https://doi.org/10.1016/0035-9203(46)90076-4).
- Protopopoff, N., Mosha, J. F., Lukole, E., Charlwood, J. D., Wright, A., Mwalimu, C. D., & Manjurano, A. (2018). Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled,

- two-by-two fact. *The Lancet*, 391(10130), 1577–1588. [https://doi.org/10.1016/S0140-6736\(18\)30427-6](https://doi.org/10.1016/S0140-6736(18)30427-6).
- Protopopoff, N., Verhaeghen, K., Van Bortel, W., Roelants, P., Marcotty, T., Baza, D., D'Alessandro, U., & Coosemans, M. (2008). A significant increase in kdr in *Anopheles gambiae* is associated with an intensive vector control intervention in Burundi highlands. *Tropical Medicine and International Health*, 13(12), 1479–1487. <https://doi.org/10.1111/j.1365-3156.2008.02164.x>.
- R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. <http://www.r-project.org/index.html>.
- Rabinovich, R. N., Drakeley, C., Djimde, A. A., Hall, B. F., Hay, S. I., Hemingway, J., Kaslow, D. C., Noor, A., Okumu, F., Steketee, R., Tanner, M., Wells, T. N. C., Whittaker, M. A., Winzeler, E. A., Wirth, D. F., Whitfield, K., & Alonso, P. L. (2017). malERA: An updated research agenda for malaria elimination and eradication. *PLoS Medicine*, 14(11), e1002456. <https://doi.org/10.1371/journal.pmed.1002456>.
- Ranson, H., & Lissenden, N. (2016). Insecticide Resistance in African *Anopheles* Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends in Parasitology*, 32(3), 187–196. <https://doi.org/10.1016/j.pt.2015.11.010>.
- Russell, T. L., Lwetoijera, D. W., Maliti, D., Chipwaza, B., Kihonda, J., Charlwood, J. D., Smith, T. A., Lengeler, C., Mwanyangala, M. A., Nathan, R., Knols, B. G., Takken, W., & Killeen, G. F. (2010). Impact of promoting longer-lasting insecticide treatment of bed nets upon malaria transmission in a rural Tanzanian setting with pre-existing high coverage of untreated nets. *Malaria Journal*, 9(1), 187. <https://doi.org/10.1186/1475-2875-9-187>.
- Sahu, S. S., Gunasekaran, K., Vanamail, P., & Jambulingam, P. (2011). Seasonal prevalence & resting behaviour of *Anopheles minimus* theobald & *An. fluviatilis* James (Diptera: Culicidae) in east-central India. *Indian Journal of Medical Research*, 133(6), 655–661.
- Scott, J. A., Brogdon, W. G., & Collins, F. H. (1993). Identification of Single Specimens of the *Anopheles gambiae* Complex by the Polymerase Chain Reaction. *The American Journal*

- of Tropical Medicine and Hygiene*, 49(4), 520–529. <https://doi.org/10.4269/ajtmh.1993.49.520>.
- Selemani, M., Msengwa, A. S., Mrema, S., Shamte, A., Mahande, M. J., Yeates, K., Mbago, M. C. Y., & Lutambi, A. M. (2016). Assessing the effects of mosquito nets on malaria mortality using a space time model: a case study of Rufiji and Ifakara Health and Demographic Surveillance System sites in rural Tanzania. *Malaria Journal*, 15(1), 257. <https://doi.org/10.1186/s12936-016-1311-9>.
- Sharp, B. L., Streat, E., Morris, N., & Kunene, S. (2007). Seven years of regional malaria control collaboration - Mozambique, South Africa, and Swaziland. *The American Journal of Tropical Medicine and Hygiene*, 76(1), 42–47.
- Sherrard-Smith, E., Skarp, J. E., Beale, A. D., Fornadel, C., Norris, L. C., & Moore, S. J. (2019). Mosquito feeding behavior and how it influences residual malaria transmission across Africa. *Proceedings of the National Academy of Sciences of the United States of America*, 116(30), 15086–15095. <https://doi.org/10.1073/pnas.1820646116>.
- Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., & Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasites & Vectors*, 5(1), 69. <https://doi.org/10.1186/1756-3305-5-69>.
- Smith, A. (1955). The distribution of resting *A. gambiae* Giles and *A. funestus* Giles in circular and rectangular mud walled huts on Ukara Island, Tanganyika. *East African Medical Journal*, 32(8), 325–329. <http://www.ncbi.nlm.nih.gov/pubmed/13261932>.
- Smith, A. (1962a). Studies on domestic habits of *Anopheles gambiae* that affect its vulnerability to insecticides. *East African Medical Journal*, 39(1), 15–24.
- Smith, A. (1962b). The preferential indoor resting habits of *Anopheles gambiae* in the Umubugwe Area of Tanganyika. *East African Medical Journal*, 39(11), 631–635.
- Stump, A. D., Atieli, F. K., Vulule, J. M., & Besansky, N. J. (2004). Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of *Anopheles gambiae* in



- response to insecticide-treated bed net trials. *The American Journal of Tropical Medicine and Hygiene*, 70(6), 591–596.
- The malERA Consultative Group on Vector Control. (2011). A research agenda for malaria eradication: Vector control. *PLoS Medicine*, 8(1), e1000401. <https://doi.org/10.1371/journal.pmed.1000401>.
- The malERA Refresh Consultative Panel on Tools for Malaria Elimination. (2017). malERA: An updated research agenda for diagnostics, drugs, vaccines, and vector control in malaria elimination and eradication. *PLoS Medicine*, 14(11), e1002455. <https://doi.org/10.1371/journal.pmed.1002455>.
- The PMI VectorLink Project. (2019). *2018/2019 Tanzania End of Spray Report*. Abt Associates.
- Tiono, A. B., Ouédraogo, A., Ouattara, D., Bougouma, E. C., Coulibaly, S., Diarra, A., Faragher, B., Guelbeogo, M. W., Grisales, N., Ouédraogo, I. N., Ouédraogo, Z. A., Pinder, M., Sanon, S., Smith, T., Vanobberghen, F., Sagnon, N., Ranson, H., & Lindsay, S. W. (2018). Efficacy of Olyset Duo, a bednet containing pyriproxyfen and permethrin, versus a permethrin-only net against clinical malaria in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomised controlled trial. *The Lancet*, 392(10147), 569–580. [https://doi.org/10.1016/S0140-6736\(18\)31711-2](https://doi.org/10.1016/S0140-6736(18)31711-2).
- Tirados, I., Costantini, C., Gibson, G., & Torr, S. J. (2006). Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: Implications for vector control. *Medical and Veterinary Entomology*, 20(4), 425–437. <https://doi.org/10.1111/j.1365-2915.2006.652.x>.
- Tusting, L. S., Bisanzio, D., Alabaster, G., Cameron, E., Cibulskis, R., Davies, M., Flaxman, S., Gibson, H. S., Knudsen, J., Mbogo, C., Okumu, F. O., von Seidlein, L., Weiss, D. J., Lindsay, S. W., Gething, P. W., & Bhatt, S. (2019). Mapping changes in housing in sub-Saharan Africa from 2000 to 2015. *Nature*, 568(7752), 391–394. <https://doi.org/10.1038/s41586-019-1050-5>.
- Tusting, L. S., Bottomley, C., Gibson, H., Kleinschmidt, I., Tatem, A. J., Lindsay, S. W., & Gething, P. W. (2017). Housing improvements and malaria risk in sub-Saharan Africa: a

- multi-country analysis of survey data. *PLoS Medicine*, 14(2), e1002234. <https://doi.org/10.1371/journal.pmed.1002234>.
- Tusting, L. S., Ippolito, M. M., Willey, B. A., Kleinschmidt, I., Dorsey, G., Gosling, R. D., & Lindsay, S. W. (2015). The evidence for improving housing to reduce malaria: A systematic review and meta-analysis. *Malaria Journal*, 14(1), 209. <https://doi.org/10.1186/s12936-015-0724-1>.
- U.S. President's Malaria Initiative. (2019a). *Indoor Residual Spraying*. PMI. <https://www.pmi.gov/how-we-work/technical-areas/indoor-residual-spraying>.
- U.S. President's Malaria Initiative. (2019b). *PMI 13th Annual Report to Congress* (Issue May). <https://www.pmi.gov/about/pmi-annual-report-2019>.
- WHO. (2012). *Global plan for insecticide resistance management in malaria vectors*. World Health Organization.
- WHO. (2015a). *An Operational Manual for Indoor Residual Spraying (IRS) for Malaria Transmission Control and Elimination*. World Health Organization.
- WHO. (2015b). *Global technical strategy for malaria 2016–2030*. World Health Organization.
- WHO. (2018a). *High burden to high impact: A targeted malaria response*. World Health Organization.
- WHO. (2018b). *World Malaria Report*. World Health Organization.
- WHO. (2019a). *Prequalification Vector Control*. <https://www.who.int/pq-vector-control/prequalified-lists/en/>.
- WHO. (2019b). *World Malaria Report 2019*. World Health Organization. <https://www.who.int/publications-detail/world-malaria-report-2019>.
- Wirtz, R. A., Zavala, F., Charoenvit, Y., Campbell, G. H., Burkot, T. R., Schneider, I., Esser, K. M., Beaudoin, R. L., & Andre, R. G. (1987). Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bulletin of the World*

*Health Organization*, 65(1), 39–45.

World Weather Online. (2019). *Morogoro Monthly Climate Averages*.  
<https://www.worldweatheronline.com/morogoro-weather-averages/morogoro/tz.aspx>.